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CHEMICAL SYNTHESIS

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Studies in the Investigation of Natural Organic Products

BY

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PREFACE

In this work I have attempted to describe the more important investigations which have been made by the organic chemist in modern times in the domain of natural organic products. The book covers only a limited field of organic chemistry, and even this is but partially surveyed.

During recent years the study of natural organic products has attracted chemists in increasing numbers. The results already obtained have opened out almost infinite possibilities and have brought us face to face with new modes of chemical reaction about which we know very little as yet.

On account of the nature of the subject-matter and the fact that the book is intended for those who have a knowledge of organic chemistry at least equal to that required for a pass B.Sc. degree, the usual textbook arrangement has not been adopted.

I am indebted to Messrs. Merck, Schimmel & Co., and particularly to the Wellcome Research Bureau, for information which they have kindly placed at my disposal.

Also, my thanks are due to Professor R. M. Caven, the general editor of the series, for many valuable suggestions, and to my chief, Mr. W. Rintoul, F.I.C., Head of the Nobel Research Laboratories, for the interest which he has taken in the preparation of the book. Finally, I am indebted to my wife for her help in preparing the book for the press.

H. HEPWORTH.

THE RESEARCH LABORATORIES, ARDEER,
March, 1924.

EDITOR'S NOTE

After many years of neglect chemistry is at length being recognized in this country as one of the most important factors of modern life. Evidence of this recognition is shown by the increased notice which the subject is receiving in the public press, and by the large numbers of books that are being issued on various aspects of the science. It is now clearly seen that chemical science contributes to the health, comfort, luxury, and intellectual life of the modern citizen. It is a difficult task, nevertheless, to bring the latest achievements of chemistry within the reach of minds untutored in its first principles; though this task should be attempted by those best qualified to perform it.

The purpose of the present series of manuals, however, is to provide for those who have a working knowledge of chemistry—for graduates in science and medicine, for workers in various branches of applied chemistry, for teachers, and for all who have an intellectual interest in the science for its own sake—readable accounts of modern developments written by experts in the subjects with which they deal.

Organic chemistry as a science is not yet one hundred years old; it is still four years to the centenary of the synthesis of urea by Wöhler; yet how amazing has been the advance in our knowledge during ninety-six years! Having started with the study of natural products, the exponents of this branch of chemistry, by elaborating artificial derivatives of the hydrocarbons, have deviated far from the path pursued by Nature herself. Nevertheless throughout this period there have always been chemists to whom the natural products of the plant and animal world themselves proved the main attraction, chemists who studied these products because they were part of Nature, irrespective of whether the study would bring any kind of gain beyond the knowledge itself. Of the labours and discoveries

of these chemists, Dr. Hepworth gives an account in the present volume. This account begins where Nature begins, with the photosynthesis of plant products; it continues with the study of chlorophyll and other natural pigments, and of the formation of carbohydrates, tannins, oils, fats, and waxes. After notice of the terpenes, and then of the amino acids and the natural bases which are elaborated from these, the volume concludes with an account of the alkaloids, and of recent research upon their synthesis. Thus the work of Nature in the organic field, and the efforts of man to follow her and elucidate her methods, are brought to view.

R. M. CAVEN.

ROYAL TECHNICAL COLLEGE, GLASGOW,
March, 1924.

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TABLE OF ABBREVIATIONS EMPLOYED IN THE REFERENCES

Abbreviated Title.	Journal.
Absts.	Abstracts in Journal of the Chemical Society, London.
Amer. Chem. J.	American Chemical Journal.
Amer. J. Physiol.	American Journal of Physiology.
Ann.	Justus Liebig's Annalen der Chemie.
Ann. Chim.	Annales de Chimie.
Arch. Pharm.	Archiv der Pharmazie.
Ber.	Berichte der Deutschen chemischen Gesellschaft.
Ber. Deut. bot. Ges.	Berichte der Deutschen botanischen Gesellschaft.
Ber. Deut. pharm. Ges.	Berichte der Deutschen pharmazeutischen Gesellschaft.
Biochem. J.	The Biochemical Journal.
Biochem. Z.	Biochemische Zeitschrift.
Bull. Soc. chim.	Bulletin de la Société chimique de France.
Chem. Zeit.	Chemiker Zeitung.
Chim. et Ind.	Chimie et Industrie.
C. r.	Comptes rendus hebdomadaires des Séances de l'Académie des Sciences.
D.R.P.	Deutsches Reichs Patent.
Gazz.	Gazzetta chimica italiana.
Helv. Chim. Acta.	Helvetica Chimica Acta.
J. Amer. Chem. Soc.	Journal of the American Chemical Society.
J. Biol. Chem.	Journal of Biological Chemistry.
J. Ind. Eng. Chem.	Journal of Industrial and Engineering Chemistry.
J. Physiol.	Journal of Physiology.
J. pr. Chem.	Journal für praktische Chemie.
J. Russ. Phys. Chem. Soc.	Journal of the Physical and Chemical Society of Russia.
J. Soc. Chem. Ind.	Journal of the Society of Chemical Industry.
Proc.	Proceedings of the Chemical Society, London.
Proc. Roy. Soc.	Proceedings of the Royal Society.
Sitz. Preuss. Akad. Wiss. Berlin.	Sitzungsberichte der Preussischen Akademie der Wissenschaften zu Berlin.
Trans.	Transactions of the Chemical Society, London.
Z. Biol.	Zeitschrift für Biologie.
Zentr.	Chemisches Zentralblatt.
Z. physiol. Chem.	Hoppe-Seyler's Zeitschrift für physiologische Chemie.

INTRODUCTION

As early as the second half of the eighteenth century, Lemery divided substances, according to their origin, into three classes, viz. mineral, vegetable, and animal, and thereby separated inorganic from organic chemistry. Even down to the third decade of the nineteenth century an important distinction was drawn between organic and mineral substances. It was supposed that the latter alone were producible artificially, while the synthesis of the former was wholly beyond the power of the chemist and was reserved for the living organism, in which it was performed under the influence of a Vital Force.

It is easy to understand why in its early youth organic chemistry was so closely connected with biology, for the materials which the chemist was called upon to investigate were mostly products of animal or vegetable origin. The isolation of urea from animal urine by Rouelle, the recognition of uric acid, lactic acid, malic acid, and glycerine by Scheele, the isolation of asparagine by Vauquelin and Robiquet, of morphine by Sertürner, together with many other similar discoveries during the first ten years of the nineteenth century, are admirable examples of the manner in which the living world was drawn upon and made to yield up its treasure of chemical compounds.

It is generally conceded that the doctrine of a special vital force was discredited as an outcome of the synthesis of urea, from lead cyanate and ammonium chloride, by Wöhler in 1828;* and as, year by year, new synthetic products were added to the list of organic compounds, this last barrier, which separated organic from inorganic

* It is not a little remarkable that John Davy had obtained urea several years before this by the action of carbonyl chloride on ammonia, but had not recognized it.

chemistry, was swept away, and henceforth the former became the chemistry of carbon compounds.

The subsequent development of organic chemistry cannot be traced here, and it will be sufficient to say that, in the form in which it exists to-day, organic chemistry may be deemed to have begun with the work of Frankland at the middle of the nineteenth century. Once the doctrine of the constancy of valency was accepted, Couper, Crum Brown, and Kekulé were able to bring order into the vast mass of material which had already been accumulated. At a later date, van't Hoff and Le Bel extended existing ideas of molecular arrangement into three dimensions, and laid the foundations of our present views. Thus it came about, as a result of the enormous theoretical and practical developments which followed these discoveries, that organic chemistry became separated from biology in the latter half of last century. The number of natural organic products enumerated in the text-books is indeed small in comparison with the 150,000 carbon compounds of which organic chemistry can boast to-day.

With the dawn of the twentieth century, more attention has been paid to the chemistry of other vital products, with a view to the elucidation of the mechanism by which these substances are elaborated by the plant and animal. This return to the field of early studies is due principally, but by no means exclusively, to the pioneering researches of Emil Fischer; and the prospects which have been opened out seem almost infinite in variety.

We know that in nature the construction of organic compounds begins with the carbon dioxide and nitrogen of the atmosphere, and the study of the mechanism by which the plant can assimilate these substances, with the ultimate production of the most complex organic compounds, is now attracting chemists in increasing numbers.

The investigation of plant pigments and especially chlorophyll—the plant pigment "par excellence"—has been the subject of a series of brilliant researches by Willstätter, and these studies have led to the development of an extraordinary technique in the use of solvents. Several points with regard to the structure of chlorophyll

have still to be cleared up, but these must apparently await further investigation of the pyrrole derivatives.

In the phytochemical * synthesis of plant products, the sugars make their appearance at a very early stage. Since 1886, the investigation of these compounds has proceeded very rapidly, principally owing to the classic researches of Emil Fischer. In spite of the extensive results accomplished, we are still far from understanding all the chemical possibilities of even the monosaccharoses, and the glucose molecule is now assuming a protean character almost as wonderful as that of camphor. The recent work of Irvine and his collaborators on the di- and poly-saccharoses, especially cellulose, demands special consideration, and valuable results may be expected from the new methods which are being employed. The discovery of the methyl glucosides and acetobromoglucose may be regarded as the cardinal points in the recent studies of the synthetic glucosides. The remarkable achievements in the investigation of gallotannin are almost unsurpassed in the realm of synthetic organic chemistry, yet gallotannin represents but one of the numerous members of the tannin family, about the majority of which we know practically nothing. Similar remarks apply to the natural gums and mucilages.

The chemical reactivity of the enzymes has received considerable attention, especially at the hands of E. Fischer, H. E. and E. F. Armstrong, Bayliss, and Bourquelot, and the value of these substances as the " chemical reagents " of the organism has been repeatedly emphasized. The chemical investigation of these substances presents considerable difficulty largely owing to their colloidal nature. Willstätter has recently initiated a series of researches with a view to the ultimate determination of the constitution of the enzymes; and if this aim is eventually achieved, it may be safely said that it will outweigh even the extraordinarily brilliant researches which this chemist has already carried out.

At first sight it would appear that the chemistry of the oils, fats, and waxes could almost be regarded as a closed chapter, but the later researches of E. Fischer have shown that even such an

* φυτόν, a plant.

apparently simple problem as the preparation of the monoglycerides is in reality full of pitfalls. Of the chemical constitution of the majority of the lipins we know practically nothing as yet, and much work remains to be done before we understand these substances even from a purely chemical point of view. The statement that fats and sugars are converted in the body to carbon dioxide and water is no longer considered an all-sufficient explanation of the rôle of these substances in the animal economy. No one imagines that in the breakdown of the higher fatty acids all the carbon atoms are immediately or directly converted into carbon dioxide. Our views as to the mechanism of oxidation reactions, both in the laboratory and in the living organism, have lately undergone considerable change, and there is a good deal of evidence in favour of the view that the first stage in the oxidation of organic substances consists in the replacement of hydrogen atoms by hydroxyl groups.

Following the synthesis of camphoric acid by Komppa in 1903, and that of dipentene by Perkin, jun., in the following year, the progress in the investigation of the mono- and di-cyclic terpenes and their derivatives has been very rapid, and almost every year new compounds belonging to these classes are being isolated from natural sources. In recent years the chemistry of the caoutchoucs has attracted considerable attention, more particularly with a view to preparing synthetic rubber. Although a good deal of progress has been made, it cannot be said that a thoroughly successful product has yet been prepared synthetically. Of the sesqui-terpenes we know very little, while we are equally ignorant of the mechanism by which the plant synthesizes its essential oils.

The introduction of a new method of separating the mono-amino acids obtained in the hydrolysis of the proteins, by E. Fischer in 1901, led to numerous investigations of the nature of the amino acids present therein. After the first decade, when the technique had been worked out, investigators began to consider the losses involved in the process of isolation, and more recently Dakin and Foreman have introduced alternative methods for the isolation of certain types of amino acids derived from the hydrolysis of proteins. Fischer's investigations of many of the naturally occurring polypep-

tides have been extended by a number of chemists, notably by Abderhalden. Perhaps the most interesting polypeptide so far discovered is glutathione, which has been isolated recently by Hopkins, and this substance bids fair to revolutionize our ideas concerning the possibilities of these compounds in the living organism. In spite of the rapid progress which has been made in the study of the proteins, vast regions still remain almost untouched.

The classification of the natural organic bases is becoming an increasingly difficult problem. The alkaloids, which were easily isolated on account of their insolubility in water and their ready solubility in organic solvents, were amongst the first natural substances to attract the organic chemist. Very substantial progress has been made in the study of these compounds, perhaps the most interesting investigation of recent years being the classic study of cryptopine and protopine by W. H. Perkin, jun., and that of harmine and harmaline by the same author in collaboration with R. Robinson. Each year brings forward new alkaloids for investigation, and the field seems almost inexhaustible. Without doubt the most satisfactory theory with regard to the phytochemical synthesis of the alkaloids which has so far been put forward is that due to R. Robinson, and a consideration of his paper cannot fail to suggest numerous problems which deserve the attention of the organic chemist. The extraction of the simpler natural bases presented more difficulty on account of their solubility in water, and the necessary technique was initiated by Brieger in 1885. It is surprising to what an extent the study of ergot has resulted in the isolation of new bases which may be regarded as derived from amino acids. The chemistry of the purines has of late years received an increased stimulus from biology, and our interest in these compounds no longer centres around caffeine, theobromine, and uric acid. The study of the nucleic acids and the purine and pyrimidine bases which are contained in them has the same fascination as that which uric acid and the xanthine bases had sixty years ago. The preparation of synthetic nucleosides and nucleotides by E. Fischer must be regarded as a valuable step towards the ultimate synthesis of the nucleic acids.

It has been stated that the idea of a vital force was dispelled almost a century ago; but the chemist must bear in mind that until he has shown that his synthetic methods are identical with those of Nature, and that he can prepare natural organic compounds from materials likely to be employed by the plant and within small limits of temperature, there is just as much scope for endeavouring to penetrate Nature's methods of synthesis as there was in the days when it was believed that every organic compound required a vital force for its elaboration.

ORGANIC CHEMICAL SYNTHESIS

CHAPTER I

The Photosynthesis of Plant Products

Introduction.—The processes by means of which green plants are enabled to assimilate nitrogen and carbon have attracted the attention of chemists for a number of years, and whatever the nature of these reactions may be, they constitute, indeed, the chemical synthesis " par excellence ". Although atmospheric nitrogen has long been recognized as the ultimate source of supply of that element from which phyto-protoplasm is constructed, modern investigation has indicated that nitrogen is not drawn by the plant directly from the air, but is assimilatated in a combined state from the soil by the roots, with or without bacterial co-operation. The majority of chemists believe that the agency by which green plants are enabled to assimilate carbon is chlorophyll, operating under solar influence by some such mechanism as will be indicated in the present chapter.

In 1870 Baeyer * put forward the hypothesis that the first product of plant assimilation is formaldehyde resulting from the photolysis of carbon dioxide in the presence of water, with the elimination of free oxygen:

$$CO_2 + H_2O = HCHO + O_2,$$

and that the resulting formaldehyde then polymerizes to give a hexose ($C_6H_{12}O_6$) (p. 42). This plausible hypothesis has influenced investigations on the synthetic aspects of carbon assimilation

* *Ber.*, 1870, 3, 63.

to a remarkable extent, and for many years the question of the presence of free formaldehyde in green leaves gave rise to the most contradictory answers. This particular point has lost a good deal of its original significance in view of the more recent results obtained, especially by Willstätter, and Baly and Heilbron.

Laboratory experiments on the polymerization of formaldehyde to the hexoses are usually quoted in favour of the formaldehyde hypothesis, but in this connection it is noteworthy that the evidence in favour of the view that cane sugar—a disaccharose—is the first carbohydrate synthesized by the plant seems almost conclusive.

Other authors consider that formic acid is the more likely intermediate product of early origin. Erlenmeyer was the first to make the suggestion, but it is only in recent years that renewed attention has been given to this possibility. Spoehr has shown that carbon dioxide and water are easily reduced to formic acid by means of radiant energy, and that a sugar-like product, which the plant can utilize as a foodstuff, is produced from formic acid under conditions such as may obtain in an active leaf.

The recent results obtained by Baly and Heilbron in their studies of the photochemical synthesis of nitrogen compounds from nitrates and carbon dioxide have given rise to a good deal of theoretical speculation as to the intermediate nitrogenous products which may possibly be formed in the plant, but these intermediate compounds are for the most part unknown to the organic chemist as yet. In view of our limited knowledge of the chemistry of the proteins the degree of our ignorance respecting the synthesis of nitrogenous compounds in the plant is not surprising.

Robinson's views on the phytochemical synthesis of the alkaloids will be dealt with in a later chapter (p. 237).

The Presence of Formaldehyde in the Plant and the Function of the Chlorophyll.—The presence of formaldehyde in the plant was first reported by Reinke * in 1883. Since that time many investigators have reported its presence, and these statements have been taken as evidence of the truth of Baeyer's hypothesis.

More recent investigators have suggested, however, that this formaldehyde is a degradation product of chlorophyll. Schryver † has confirmed Ewart's view ‡ that chlorophyll contains combined formaldehyde. The former investigator found that formaldehyde

* *Ber. deut. bot. Gesells.*, 1883, **1**, 406. † *Proc. Roy. Soc.*, 1910, **82** B, 226.
‡ *Ibid.*, 1908, **80** B, 30.

is more abundant in chlorophyll films after exposure to bright sunlight than when exposed to a dull light. When glass plates covered with films of chlorophyll were kept in the dark no formaldehyde was produced, even when moist carbon dioxide was present. If such plates were exposed to sunlight in an atmosphere free from carbon dioxide a very minute quantity of formaldehyde was produced, and the presence of moist carbon dioxide increased the quantity of formaldehyde very considerably. From these experiments Schryver concluded that in the presence of sunlight, water, and carbon dioxide, there is a continuous production of formaldehyde, which is being continually condensed to sugar. If this condensation does not proceed rapidly enough to remove all the formaldehyde, the excess enters into combination with the chlorophyll, to be set free later. In this way the quantity of free formaldehyde is so regulated that at no time is a toxic quantity present.

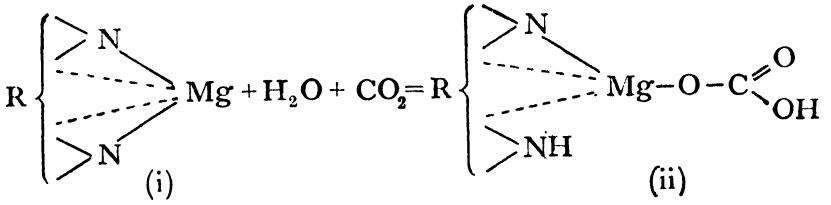
Wager * has studied the decomposition of chlorophyll on exposure to oxygen both in sunlight and in the dark, and concludes that the process is not catalytic. Oxygen is absorbed and aldehydes are formed, and it is suggested that the sugars produced during assimilation are not formed directly from carbon dioxide and water, but by the polymerization of aldehydes produced in this way. Warner † states that formaldehyde is produced when chlorophyll is exposed to sunlight in air, either in the presence or absence of carbon dioxide, from which he concludes that the latter plays no part in the production of formaldehyde by photosynthesis outside the plant, and that the formaldehyde is in reality an oxidation product of the chlorophyll.

Jörgensen and Kidd ‡ employed chlorophyll-*a* and -*b* (p. 16), and on exposing an aqueous chlorophyll sol, contained in glass vessels and in contact with various gases, to light, they found that formaldehyde was only produced in the presence of oxygen. In the case of carbon dioxide, phæophytin (p. 17) was produced and there was no further change. These authors suggest that the formaldehyde arises chiefly from the phytol (p. 15) which is probably split off from the chlorophyll under the action of light and oxygen.

These views have, however, been more recently superseded by the experiments of Willstätter and Stoll,§ who showed that no formaldehyde was formed if pure chlorophyll, in colloidal solution, was employed—the colloidal solution being considered to approximate

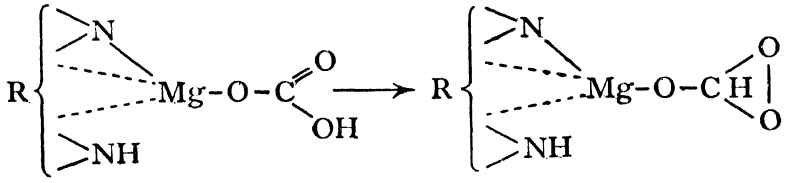
* *Proc. Roy. Soc.*, 1914, 87 B, 386. † *Ibid.*, p. 378.
‡ *Proc. Roy. Soc.*, 1916, 89 B, 342. § *Ber.*, 1917, 50, 1791.

most closely to the condition of the chlorophyll in the chloroplast. The formaldehyde described by earlier workers is attributed to the oxidation of impurities accompanying the chlorophyll they employed. The failure to obtain any trace of formaldehyde from pure chlorophyll *in vitro* is attributed to the absence of the essential enzyme present in the green leaf. Experiments *in vitro* have shown that carbon dioxide reacts with chlorophyll (i) in colloidal solution to form a compound of the nature of a bicarbonate (ii).



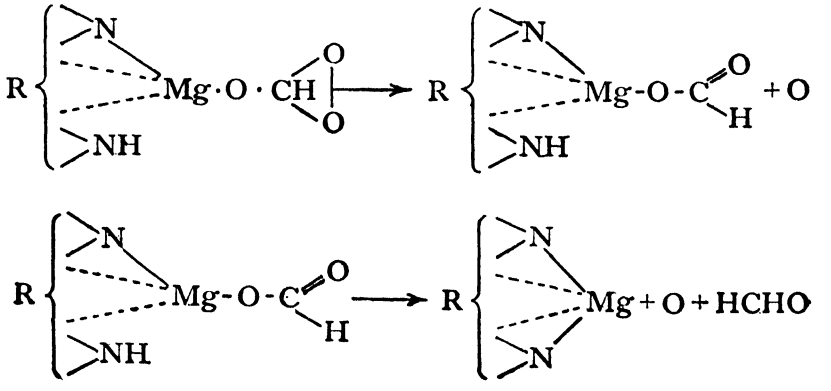
(i) (ii)

It is very unlikely that a compound of constitution (ii) would yield two atoms of oxygen with regeneration of chlorophyll, so that some intramolecular rearrangement must first take place, and this, according to Willstätter and Stoll, involves the absorption of energy, which is supplied by sunlight. In this way a formaldehyde-peroxide compound (iii) is assumed to be formed:



(iii)

This compound should be easily capable of losing oxygen, either in one or two stages, with regeneration of unaltered chlorophyll and formation of formaldehyde.



No such peroxide (iii) has been observed when experiments are carried out *in vitro*, but this is not considered surprising, " in view of the essential difference between test-tube experiments and the activity of the living cell ". The chloroplast will tolerate concentrations of carbon dioxide which decompose chlorophyll in colloidal solution to phæophytin, with precipitation of magnesium carbonate, so that the chlorophyll in the chloroplast must be protected from photo-oxidation in some way. Evidence has been adduced that within the living cell the decomposition of the peroxide-formaldehyde compound (iii) is brought about by an enzyme.

Spoehr * has shown that certain plant acids, especially dibasic acids, readily undergo decomposition when exposed to ultra-violet light in quartz vessels, with the formation of acetaldehyde and acetic acid, and that the latter may undergo further decomposition, yielding formaldehyde and formic acid.

The most satisfactory evidence that formaldehyde is the connecting link between carbon dioxide and the carbohydrates has been supplied by Willstätter and Stoll.† Of all the possible primary products, formaldehyde is the only one in the formation of which the volume of carbon dioxide absorbed would be equal to the volume of oxygen liberated. In other words, the " assimilatory quotient ", CO_2/O_2, is unity in the case of formaldehyde, 1·33 for glycollic acid, 2 for formic acid, and 4 for oxalic acid. This quotient has been determined experimentally and found to be unity, whether the temperature is 10° or 35°, whether the atmosphere is rich in carbon dioxide or even deprived of oxygen altogether, or whether ordinary foliage or succulent leaves, like cactus, are examined.

It should be pointed out that although since the days of de Saussure (1804) chlorophyll has been regarded as the fundamental agent in the photosynthesis of plant matter, there is no experimental evidence that the primary agent may not be contained in the colourless part of the chloroplast, chlorophyll thus being the result of a later synthetic stage. " The function of the chlorophyll may be a protective one to the chloroplast when exposed to light, it may be a light-screen as has been suggested by Pringsheim, or it may be concerned in condensations and polymerizations subsequent to the first act of synthesis with production of formaldehyde." ‡ In this connection it is noteworthy that in 1892 Molisch showed that

* *Biochem. Zeitsch.*, 1913, **57**, 95. † *Ber.*, 1917, **50**, 1777.
‡ *Biochemistry*, by B. Moore, p. 55.

chlorosis of green plants will follow a deficiency of iron even in the presence of sunlight and that development of chlorophyll can be restored by supplying the deficiency, although iron is not a component of the chlorophyll molecule. Green leaves etiolated by darkness and then exposed to light regain their chlorophyll, which is therefore itself a product arising from photosynthesis.

Photocatalysis: the Synthesis of Formaldehyde from Carbon Dioxide and Water.—It is a well established fact that an aqueous solution of carbon dioxide is unable to absorb visible light, but that it absorbs ultra-violet light of extremely short wave-length. In order to gain the energy required for the first stage of the synthesis, the carbon dioxide and water must be exposed to light of this very short wave-length. Since sunlight includes at the most only a minute quantity of this light, the synthesis cannot be initiated by sunlight, and we have therefore to account for the fact that the plant is able to accomplish this synthesis in ordinary sunlight.

Baly and Heilbron * have suggested a theory which is based on the quantitative study of the formation of hydrogen chloride from hydrogen and chlorine.† It was found that the velocity of this reaction is not proportional to the intensity of the light, but increases far more rapidly than the intensity; i.e. the amount of hydrogen chloride formed with a given quantity of energy is not constant, but increases rapidly to an explosive maximum as the intensity of the light is increased. The reaction may be formulated:

$$H_2 + Cl_2 + E = 2HCl + E + K,$$

where E is the amount of energy absorbed in activating the chlorine molecule and K is the normal heat of formation of two molecules of hydrogen chloride. The total energy E + K is radiated at infra-red (heat) frequencies, which are characteristic of the hydrogen molecule. It has been proved, however, that many of the infra-red frequencies of a compound molecule are identical with those of its component atoms, and consequently the molecules of hydrogen chloride and chlorine have some infra-red frequencies in common. Part of the energy E + K will therefore be reabsorbed by the surrounding chlorine molecules, with the result that these become partially or wholly activated. Baly and Heilbron consider that the principle is applicable to all photochemical reactions, and may be made use of in promoting a reaction when the reactant molecules

* *Trans.*, 1921, **119**, 1025. † Baly and Barker, *Trans.*, 1921, **119**, 653.

are screened from the ultra-violet rays they normally require. For this purpose the reactants are mixed with a " photocatalyst " (A), which absorbs rays different from those characteristic of the reactants, but which has the same infra-red frequencies as the reactants. When such a mixture is exposed to rays absorbed by the substance A, the energy thus absorbed will be radiated at the infra-red frequencies characteristic of A; and since these are the same as those of the reactant molecules, the latter will reabsorb this radiation and the reaction will take place.

Now Moore and Webster * have stated that a saturated aqueous solution of carbon dioxide gives no formaldehyde on exposure to ultra-violet light, but that in the presence of certain inorganic " catalysts ", e.g. colloidal ferric hydroxide, beryllium chloride, &c., small quantities of formaldehyde are produced. Baly and Heilbron have confirmed these experiments, and in addition have observed that if a solution of carbon dioxide in water is agitated by carbon dioxide during the exposure to ultra-violet light, distinct traces of formaldehyde can soon be detected. These authors have advanced two reasons why the solution must be agitated in order to obtain positive results:

1. In ultra-violet light the liberated oxygen would combine with water to give hydrogen peroxide, which would oxidize the formaldehyde to formic acid.

2. If the solution is not agitated, the formaldehyde which escapes oxidation is polymerized as fast as it is formed, whereas agitation carries a portion of the formaldehyde to the back of the vessel, where the actinic intensity of the light is less.

These authors have found that formaldehyde is polymerized by long-wave ultra-violet light (290 $\mu\mu$), while its synthesis requires short-wave ultra-violet light (200 $\mu\mu$). Paraldehyde and sodium phenate absorb the long-wave ultra-violet light, and therefore if added to the solution protect the formaldehyde from polymerization, and it has been shown that the so-called inorganic catalysts employed by Moore and Webster behave in a similar manner.

In ultra-violet light a photo-equilibrium is established:

$$\text{Carbohydrate} \longrightarrow \text{Carbon Dioxide and Water}$$
$$\uparrow \qquad\qquad \downarrow$$
$$\text{Formaldehyde.}$$

In order that the first stage may be photocatalyzed, a substance

* *Proc. Roy. Soc.*, 1914, 87 B, 163, 556; 1918, 90 B, 168.

must be used which has the same infra-red frequencies as carbon dioxide; and malachite green, methyl orange, and *p*-nitroso-dimethylaniline have been found to be suitable photocatalysts for this reaction. A suitable photocatalyst for the second stage of the reaction has not yet been found, but these authors suggest that chlorophyll is an ideal photocatalyst for both stages of the synthesis.

The Photosynthesis of Nitrogen Compounds from Nitrates and Carbon Dioxide.—Potassium nitrate and possibly ammonium salts are the sources from which the plant derives its nitrogen, but nitrates as such are relatively inert substances which do not readily lend themselves to chemical change, whereas nitrites, on the other hand, are much more reactive.

In 1890 Laurent observed that the plant is able to convert nitrate into nitrite, and this fact was soon afterwards confirmed by other workers. As early as 1883 Schimper found that nitrates were destroyed in green leaves exposed to daylight, but were not so destroyed if the leaves were kept in the dark. Furthermore, no destruction of nitrate occurs in etiolated leaves exposed to sunlight.

Thiele * first recorded the rapid conversion of nitrate into nitrite by the rays from a mercury quartz lamp, evolution of oxygen occurring simultaneously. Baudisch † exposed mixtures of potassium nitrite and methyl alcohol in aqueous solution to diffused daylight and to ultra-violet light, and found that the methyl alcohol became oxidized to formaldehyde at the expense of the nitrite, which was reduced to hyponitrite, and the latter, at the moment of its formation, reacted with the formaldehyde to form the potassium salt of formhydroxamic acid (i):

$$KNO_2 + CH_3OH = KNO + HCHO + H_2O$$

$$KNO + HCHO = H \cdot C(=N \cdot OK) \cdot OH \quad \text{(i)}$$

No reaction took place in the dark even if the solutions were boiled, so that the change was clearly photochemical.

Moore found that, in solutions of nitrate undergoing this reduction, green leaves check the accumulation of nitrite, thus indicating their capacity to absorb the more active compound. Proceeding from the hypothesis that one of the organisms arising earliest in the course of evolution must have possessed, united in a

* *Ber.*, 1907, **40**, 4914.

† *Ber.*, 1911, **44**, 1009; 1916, **49**, 1176; 1918, **51**, 793.

single cell, the dual function of assimilating both carbon and nitrogen, Moore examined the simplest unicellular algæ. He found that in the absence of all sources of nitrogen except the atmosphere, and in presence of carbon dioxide, these algæ can fix nitrogen, grow, and form proteins by utilization of light energy. The rate of growth is accelerated by the presence of nitrites or oxides of nitrogen. Moore and Webster * have also made the important observation that the reduction of nitrates to nitrites takes place in the roots and stems where photochemical reaction is excluded.

More recently Baly, Heilbron, and Hudson † have investigated the photosynthesis of nitrogen compounds from nitrates and carbon dioxide by passing the latter through aqueous solutions of potassium nitrate or nitrite exposed to ultra-violet light. In these experiments the following observations were made:

1. Activated formaldehyde, such as is photochemically produced, reacts with potassium nitrite, and this reaction takes precedence over that in which formaldehyde is converted into sugars.

2. If formaldehyde is produced at a greater rate than it can react with the nitrite, reducing sugars are formed.

In these circumstances activated formaldehyde is assumed to have the structure H — C — OH, the activity being due to the bivalent carbon atom. It is further assumed that the first product of reaction is formhydroxamic acid (i), an atom of oxygen being evolved which oxidizes a further quantity of formaldehyde to formic acid. These changes may be represented by the following equations:

$$\mathrm{H-C-OH + O:NOK \rightarrow H\cdot C(=N\cdot OK)\cdot OH \rightarrow H\cdot C(=N\cdot OK)\cdot OH + O}$$

(i)

$$\mathrm{O + H-C-OH = H\cdot COOH}$$

Under the conditions of the experiment, the potassium salt is completely hydrolyzed to the free acid:

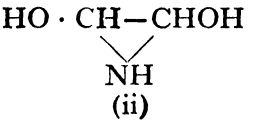
$$\mathrm{H-C(=N-OH)-OH}$$

and the latter compound readily loses oxygen with the formation of a compound:

$$\mathrm{H-C(=NH)-OH}$$

* *Proc. Roy. Soc.*, 1919, **90** B, 158. † *Trans.*, 1922, **121**, 1078.

which may be regarded as a hydrate of hydrocyanic acid. With formaldehyde the latter yields a labile ring compound (ii) which undergoes rearrangement to give glycine (iii):



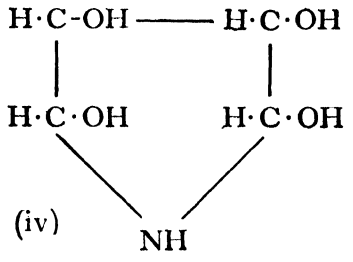
(ii)

CH_2NH_2COOH

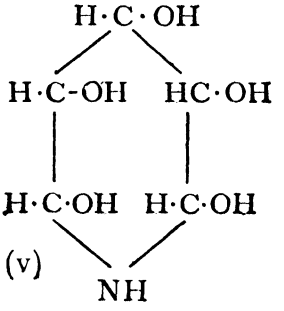
(iii)

Aqueous solutions of formhydroxamic acid—prepared by the action of ethyl formate on hydroxylamine in methyl alcoholic solution—and formaldehyde were exposed to ultra-violet light, when a reaction quickly ensued and a variety of products including methylamine and a mixture of α-amino acids (detected qualitatively only) were formed. Methylamine is probably formed directly from ammonia and formaldehyde, the latter acting as a methylating agent (p. 215).

In addition, it is claimed that alkaloids have been produced. The authors explain the formation of these substances by assuming that formhydroxamic acid condenses with three or four molecules of activated formaldehyde to produce compounds (iv) and (v) which by loss of water and oxygen give pyrrole and pyridine compounds respectively:

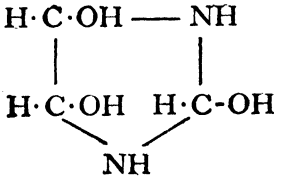


(iv)

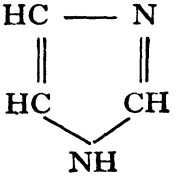


(v)

By the condensation of two molecules of formhydroxamic acid with one molecule of formaldehyde, the compound (vi) would be produced which by loss of oxygen and water would yield glyoxaline (vii) (p. 178).



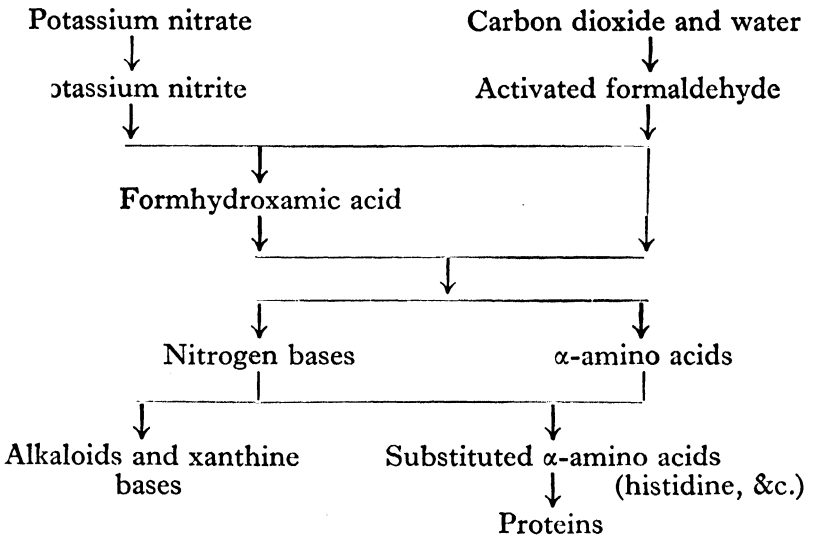
(vi)



(vii)

Evidence of the formation of this substance, as well as histidine (p. 145), has been adduced.

The authors summarize their views as follows:



The readiness with which all these reactions take place is assumed to be due to the cardinal fact that the various intermediate compounds are produced in highly reactive phases.

Still more recently Baly, Heilbron, and Stern * have obtained a number of naturally occurring nitrogen compounds photosynthetically from carbon dioxide and ammonia. Although the products of the action of light on carbonic acid and ammonia differ from those formed when carbonic acid and potassium nitrate are illuminated, the mechanism of the synthesis appears to be very similar in the two cases. During the first part of the investigation aqueous solutions of ammonia, saturated with carbon dioxide, were exposed for various periods of time to the light from a quartz mercury lamp, and the final product was found, in the main, to be methylamine. In addition, nitric and probably nitrous acids are formed. This photosynthesis is supposed to take place in two stages: first, the photosynthesis of formaldehyde by the action of light on the carbonic acid,

$$H_2CO_3 = H \cdot C \cdot OH + O_2;$$

and, secondly, the interaction of the activated formaldehyde and ammonia,

$$NH_3 + H \cdot C \cdot OH = CH_3NH_2 + O.$$

The oxidation of the ammonia to nitric acid is said to be due to the

* *Trans.*, 1923, **123**, 185.

oxygen that is set free in these two reactions. Batteries of eight quartz tubes, each containing 100 c. c. of 1·3 N-ammonia, saturated with carbon dioxide, were exposed to the light of a quartz mercury lamp for different periods, and the presence of pyridine (or piperidine) was qualitatively confirmed in every instance. Neither α-amino acids, sugars, hydroxylamine, nor hyponitrous acid was present.

By the prolonged action of ultra-violet light on 2N-ammonia and formaldehyde an alkaloid, which was thought to be conine, was obtained.

These reactions have been carried out in daylight or ultra-violet light; but it should be remembered that the synthesis of proteins can also take place in the dark and in tissues free from chlorophyll, provided that an adequate supply of carbohydrate is available. Indeed, there is good evidence in favour of the view that nitrogen assimilation is not a photochemical process, and that light is only of indirect importance in providing one of the means for the formation of carbohydrates.

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CHAPTER II

Chlorophyll and other Natural Pigments

CHLOROPHYLL

Introduction.—The rôle of chlorophyll in the realm of vital synthesis has already been dealt with in the previous chapter. For obvious reasons a correct knowledge of its constitution is of the utmost interest to the organic chemist and the botanist. The chemical study of chlorophyll dates from the year 1819, when Pelletier and Caventou first applied this name to the green-leaf pigment, without, however, isolating the substance. Since then numerous workers, amongst whom Schenck and Marchlewski may be mentioned, have attempted to prepare chlorophyll in a pure condition, but the methods employed in most cases were too drastic. Previous to 1911, there was no chemical evidence to show that chlorophyll was not a single chemical individual, although Stokes, Sorby, and others had obtained spectroscopic evidence pointing to the existence of more than one substance. During the last ten years chlorophyll has been subjected to careful chemical investigation by Willstätter and his collaborators, and, although some minor points still remain to be cleared up, our knowledge of the chemistry of chlorophyll has made enormous progress.

In 1912 Willstätter and Isler * definitely showed that chlorophyll, as ordinarily obtained, and to which they had originally assigned the formula $C_{55}H_{72}O_6N_4Mg$, is in reality a mixture of two substances, and that two yellow pigments, carotin and xanthophyll, accompany chlorophyll in the chloroplast of plants. A further pigment, fucoxanthin, has been isolated from brown algæ.

The presence of magnesium in chlorophyll is remarkable, and at once suggests other complex organic compounds containing traces

* *Ann.*, 1912, **390**, 269.

of metallic elements. Of these the best known is hæmoglobin, the red colouring matter of blood, which contains iron, and yields by chemical decomposition compounds having a fundamental structure similar to those obtained from chlorophyll. Hæmocyanine, the main constituent of the blood of the octopus, contains 0·38 per cent of copper, while, according to Church, the red colour exhibited by a number of African birds called turacos is due to a pigment turacine, which contains 8 per cent of copper.

Extraction of Plant Pigments.—The usual method of extracting chlorophyll from green tissues consists in first steeping the fresh material in hot water to destroy oxidizing enzymes, and then extracting the colouring matter with warm alcohol. Willstätter has shown that the operation of drying produces no change of importance in the chlorophyll, and he accordingly recommends the use of dried in place of fresh material, and extraction by shaking with organic solvents (ethyl or methyl alcohol, ether, or acetone) in the cold. Organic solvents containing an appreciable amount of water are preferable to dry solvents.

When cold alcohol is used for the extraction the so-called " crystalline chlorophyll " is obtained, whereas with ether an " amorphous chlorophyll " results.* The yellow pigments may be eliminated by a regulated system of partition among solvents, finally making use of the insolubility of chlorophyll in petroleum ether. The green pigments may also be saponified by alkalies, and are then insoluble in ether. This method can be adopted to separate the green from the yellow pigments. The separation of carotin from xanthophyll is based on the fact that when a mixture of petrol and methyl alcohol containing a little water is employed, the carotin passes into the petrol, whereas the greater part of the xanthophyll remains in the methyl alcohol layer. The distribution of the pigments in plants may be roughly summarized thus:

Green pigments	Chlorophyll-*a*, 2 parts per 1000.
	Chlorophyll-*b*, $\frac{3}{4}$ part per 1000.
Yellow pigments	Carotin, $\frac{1}{6}$ part per 1000.
	Xanthophyll, $\frac{1}{3}$ part per 1000.

The common Nettle (Urtica) is the plant which has most frequently been used for the extraction of chlorophyll on a large scale.

Amorphous and " Crystalline " Chlorophyll.—Willstätter

* Willstätter and Benz, *Ann.*, 1908, 358, 267.

and his collaborators obtained specimens of amorphous chlorophyll from upwards of two hundred plants drawn from numerous cryptogams and phanerogams. The formula suggested for this chlorophyll was $C_{55}H_{72}N_4O_5Mg$, and on decomposition all the samples yielded about 30 per cent of an alcohol named phytol.* Hydrolysis with cold, dilute, caustic potash gave equimolecular quantities of methyl alcohol, phytyl alcohol, and a tribasic acid called chlorophyllin.† Amorphous chlorophyll therefore appears to be the di-ester of a tribasic acid. Since only five oxygen atoms are present in amorphous chlorophyll there cannot be more than two carboxyl groups present. Internal anhydride formation is precluded, since phytochlorin-*e*, a decomposition product of chlorophyll, contains the same grouping and does not form an amide with ammonia.‡ No amide is present, since chlorophyll gives no ammonia on hydrolysis, and it was finally suggested that the fifth oxygen atom forms part of a lactam ring.

$$C_{31}H_{30}N_3Mg\begin{cases} =N \\ | \\ -CO \\ -COOCH_3 \\ -COOC_{20}H_{39} \end{cases}$$

Amorphous chlorophyll

$$+\ 3KOH \xrightarrow{\text{Hydrolysis}} C_{31}H_{30}N_3Mg\begin{cases} =NH \\ -COOK \\ -COOK \\ -COOK \end{cases} + CH_3OH + C_{20}H_{39}OH$$

Chlorophyllin salt — Phytol

Crystalline chlorophyll was found to be a di-ester which contained an ethyl group in the place of the phytyl radicle, while the second carboxyl group was esterified with methyl alcohol and the third carboxyl group resembled that of amorphous chlorophyll. Willstätter and Stoll § found that during the extraction with alcohol an enzyme " chlorophyllase " is set free, and this brings about the substitution. In other respects amorphous and crystalline chlorophyll are probably identical.

Phytol.—According to the investigations of Willstätter, Schuppli, and Mayer ‖ phytol is a primary alcohol of the composition $C_{20}H_{39}OH$. On oxidation with chromic acid a ketone, $C_{17}H_{34}O$,

* Willstätter and Oppé, *Ann.*, 1911, 378, 1. † *Ann.*, 1910, 378, 18.
‡ Willstätter and Utzinger, *Ann.*, 1911, 382, 129.
§ *Ann.*, 1910, 378, 18. ‖ *Ann.*, 1919, 418, 121.

is obtained, from which it is concluded that phytol contains a double bond between the α and β carbon atoms of the chain (i).

$$\begin{array}{ccc} (\beta) & & (\alpha) \\ C_{15}H_{31}C & = & C \cdot CH_2OH \\ | & & | \\ CH_3 & & CH_3 \end{array}$$

(i)

On further oxidation phytol gives phylenic acid and this acid forms a lactone. Such behaviour is characteristic of acids containing methyl groups in the α and β positions, and a double bond between the α and β carbon atoms.

Chlorophyll-*a* and Chlorophyll-*b*.—It has already been mentioned that as early as 1864 Stokes obtained spectroscopic evidence pointing to the existence of more than one substance in chlorophyll. The latter was separated into two substances termed chlorophyll-*a* and chlorophyll-*b* by Willstätter in 1912.* Chlorophyll-*a* is a blue-black solid giving a green-blue solution in organic solvents. Chlorophyll-*b* is a green-black solid giving a green solution. The two chlorophylls have much the same solubility in the common organic solvents, but can be separated by their different solubilities in methylalcohol. Both can be obtained in microscopic crystals. These chlorophylls may be written:

$(C_{32}H_{30}ON_4Mg)(COOCH_3)(COOC_{20}H_{39})$ Chlorophyll-*a*
$(C_{32}H_{28}O_2N_4Mg)(COOCH_3)(COOC_{20}H_{39})$ Chlorophyll-*b*.

Nomenclature.—The magnesium atoms of either of the chlorophylls may be removed and replaced by two hydrogen atoms, with the aid of alcoholic oxalic acid. In this reaction the ester groups remain intact, and the hydrogen derivative is known as a phæophytin.† The reverse change, i.e. the replacement of hydrogen by magnesium, can be carried out by means of the Grignard reagent.‡

When hydrolysis is allowed to proceed until the phytyl § radicle is removed, the monomethylester which remains is called a phæophorbide;|| while the removal of the methyl radicle leaves a dibasic acid phæophorbin; e.g.

* *Ann.*, 1912, **390**, 269. † From Gr., φαιός = dusky.
‡ *Ann.*, 1913, **396**, 180. § From Gr., φυτόν = a plant.
|| From Gr., φορβή = food.

$$[C_{32}H_{30}ON_4Mg]\begin{matrix}COOCH_3\\COOC_{20}H_{39}\end{matrix} \xrightarrow[+H_2]{-Mg} [C_{32}H_{32}ON_4]\begin{matrix}COOCH_3\\COOC_{20}H_{39}\end{matrix}$$

Chlorophyll-*a* — Phæophytin-*a* (Phytyl phæophorbide)

$$\xrightarrow{\text{Hydrolysis}} [C_{32}H_{32}ON_4]\begin{matrix}COOCH_3\\COOH\end{matrix} \xrightarrow{\text{Hydrolysis}} [C_{32}H_{32}ON_4]\begin{matrix}COOH\\COOH\end{matrix}$$

Phæophorbide-*a* — Phæophorbin-*a*

The chlorophyll derivatives may be classified in two main groups according as they contain magnesium or are derived from the latter by replacing the magnesium by hydrogen.

Magnesium Derivatives.	Corresponding Hydrogen Compounds.
Chlorophyll, $MgR\begin{cases}COOCH_3\\COOC_{20}H_{39}\\COOH\end{cases}$	Phæophytin, $H_2R\begin{cases}COOCH_3\\COOC_{20}H_{39}\\COOH\end{cases}$
Chlorophyllide, $MgR\begin{cases}COOCH_3\\(COOH)_2\end{cases}$	Phæophorbide, $H_2R\begin{cases}COOCH_3\\(COOH)_2\end{cases}$
Chlorophyllin, $MgR(COOH)_3$	Phæophorbin, $H_2R(COOH)_2$
Glaucophyllin* or Cyanophyllin, $MgRH(COOH)_2$	Glaucoporphyrin† or Cyanoporphyrin, $H_2RH(COOH)_2$
Pyrophyllin, $MgRH_2(COOH)$	Pyroporphyrin, $H_2RH_2(COOH)$
Ætiophyllin, $MgRH_3$	Ætioporphyrin, H_2RH_3

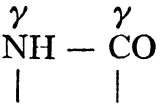
In the tricarboxylic derivatives one carboxyl group is, of course, masked by lactam formation.

Action of Alkalies and Acids on Chlorophyll.—It has already been stated that when chlorophyll-*a* is treated with alkali at ordinary temperature the phytyl radical is displaced, that a methyl group is then eliminated, and that finally a tribasic acid, chlorophyllin-*a*, is produced. At 140° carbon dioxide is split off and glaucophyllin, a dicarboxylic acid, is obtained. At 165° rearrangement occurs and rhodophyllin is produced, which in turn at 200° loses carbon dioxide, with the production of a monocarboxylic acid, pyrophyllin.‡

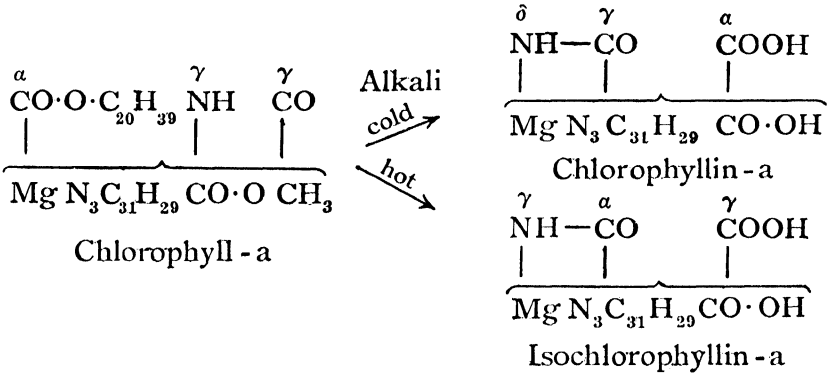
If hot alkali is allowed to react with chlorophyll-*a*, an intramolecular change occurs with the production of isochlorophyllin-*a*, someric with chlorophyllin-*a*. In this reaction the green colour

* From Gr., γλαῦκος = bluish green. † From Gr., πορφύρεος = purple.
‡ Willstätter and Fritzsche, *Ann.*, 1909, **371**, 33.

changes to a yellowish-brown but, after a few minutes, the original green colour reappears. Willstätter * has suggested that this phenomenon is due to the presence of the lactam group which opens and re-forms in a new position. The original lactam group may be denoted:



On hydrolysis the carboxyl γ may enter into union with another nitrogen group δ, or another carboxyl α may combine with nitrogen γ. The action of cold and hot alkali may be represented by the scheme:



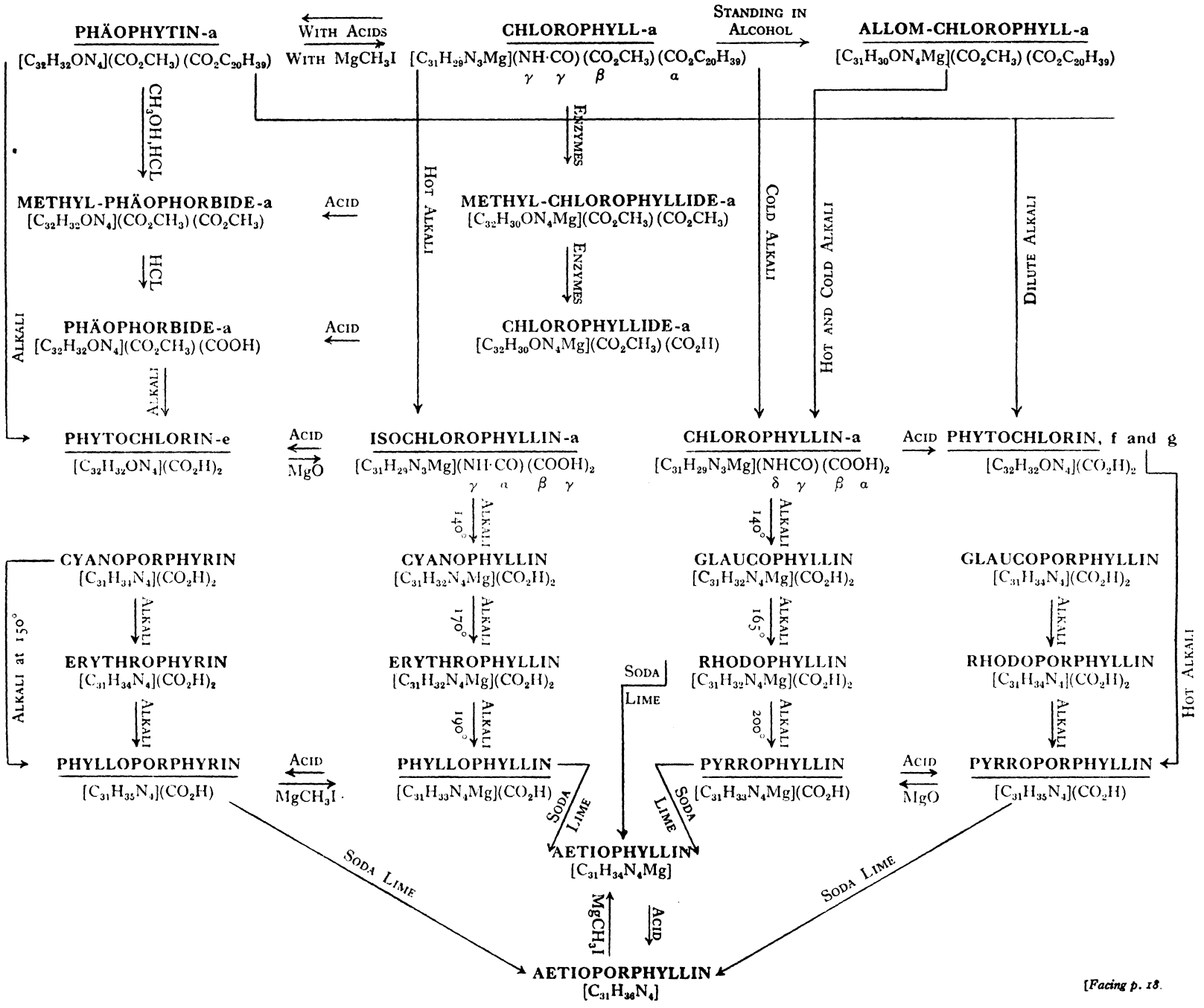
Willstätter assumes that at least three of the nitrogen atoms of chlorophyll are capable of taking part in lactam ring formation, and since there are three carboxyl radicles also present in the molecule it can be seen that a very considerable number of lactams may be formed. This type of rearrangement has been termed " allomerization " by Willstätter.

The further action of alkali, at higher temperatures, on chlorophyll-*a* results in the successive formation of cyanophyllin, erythrophyllin, and phyllophyllin, which are isomeric with glaucophyllin, rhodophyllin, and pyrophyllin respectively.

By the action of acids on chlorophyll, the magnesium is removed from the molecule and replaced by two hydrogen atoms. Similar results are obtained by the action of acids on the decomposition products of chlorophyll, so that for each magnesium derivative there is a corresponding hydrogen compound.

The phyllins and porphyrins when heated with soda-lime are

* *Ann.*, 1911, 382, 129.


PHÄOPHYTIN-a
[C32H32ON4](CO2CH3)(CO2C20H39)
WITH ACIDS
WITH MgCH3I
CHLOROPHYLL-a
[C31H29N3Mg](NH·CO)(CO2CH3)(CO2C20H39)
γ γ β α
STANDING IN ALCOHOL
ALLOM-CHLOROPHYLL-a
[C31H30ON4Mg](CO2CH3)(CO2C20H39)
CH3OH,HCl
ENZYMES
METHYL-PHÄOPHORBIDE-a
[C32H32ON4](CO2CH3)(CO2CH3)
ACID
METHYL-CHLOROPHYLLIDE-a
[C32H30ON4Mg](CO2CH3)(CO2CH3)
HOT ALKALI
COLD ALKALI
HOT AND COLD ALKALI
DILUTE ALKALI
HCl
ENZYMES
ALKALI
PHÄOPHORBIDE-a
[C32H32ON4](CO2CH3)(COOH)
ACID
CHLOROPHYLLIDE-a
[C32H30ON4Mg](CO2CH3)(CO2H)
ALKALI
PHYTOCHLORIN-e
[C32H32ON4](CO2H)2
ACID
MgO
ISOCHLOROPHYLLIN-a
[C31H29N3Mg](NH·CO)(COOH)2
γ α β γ
CHLOROPHYLLIN-a
[C31H29N3Mg](NHCO)(COOH)2
δ γ β α
ACID
PHYTOCHLORIN, f and g
[C32H32ON4](CO2H)2
140°
ALKALI
CYANOPORPHYRIN
[C31H31N4](CO2H)2
CYANOPHYLLIN
[C31H32N4Mg](CO2H)2
GLAUCOPHYLLIN
[C31H32N4Mg](CO2H)2
GLAUCOPORPHYLLIN
[C31H34N4](CO2H)2
ALKALI AT 150°
ALKALI
170°
165°
ERYTHROPHYRIN
[C31H34N4](CO2H)2
ERYTHROPHYLLIN
[C31H32N4Mg](CO2H)2
SODA LIME
RHODOPHYLLIN
[C31H32N4Mg](CO2H)2
RHODOPORPHYLLIN
[C31H34N4](CO2H)2
190°
200°
PHYLLOPORPHYRIN
[C31H35N4](CO2H)
ACID
MgCH3I
PHYLLOPHYLLIN
[C31H33N4Mg](CO2H)
PYRROPHYLLIN
[C31H33N4Mg](CO2H)
ACID
MgO
PYRROPORPHYLLIN
[C31H35N4](CO2H)
HOT ALKALI
SODA LIME
SODA LIME
SODA LIME
AETIOPHYLLIN
[C31H34N4Mg]
MgCH3I
ACID
AETIOPORPHYLLIN
[C31H36N4]

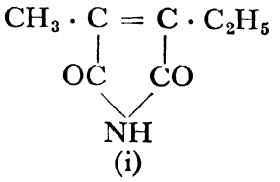
[*Facing p. 18.*

converted into ætiophyllin, $C_{31}H_{34}N_4Mg$, and ætioporphyrin, $C_{31}H_{36}N_4$, respectively.

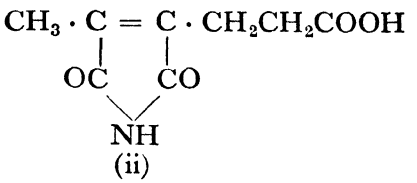
The table facing p. 18 summarizes the various decomposition products of chlorophyll-*a*. Some of the intermediate products formed by the decomposition of chlorophyll-*a* do not appear to be formed when chlorophyll-*b* is similarly treated.*

Ætiophyllin and Ætioporphyrin.—The ætiophyllin molecule contains no oxygen, so that the magnesium atom is probably attached to nitrogen.

By the oxidation of phylloporphyrin, methyl-ethyl-maleinimide (i) and hæmatinic acid (ii) are formed. The structures of these substances are known with certainty to be:

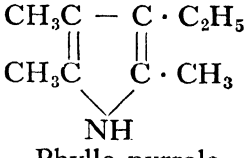


(i)

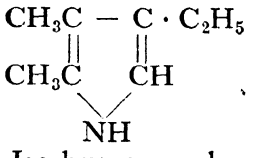


(ii)

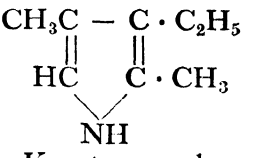
Nencki and Marchlewski observed the production of hæmopyrrole on the reduction of the porphyrins. Hæmopyrrole is a mixture of three pyrrole derivatives, namely, an ethyl trimethyl and two isomeric dimethylethylpyrroles of the following structures:



Phyllo-pyrrole

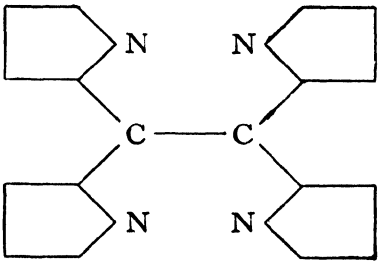


Iso-hæmopyrrole



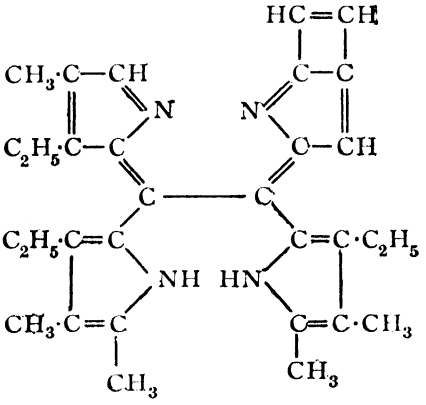
Krypto-pyrrole

Willstätter concludes that ætioporphyrin is probably a compound of four pyrrole nucleii. Owing to the deficiency in hydrogen, these nucleii must be so combined and substituted by double bonds or the formation of rings that eight atoms of hydrogen less are required than if single bonds were present. These considerations lead to a skeleton framework of the type:

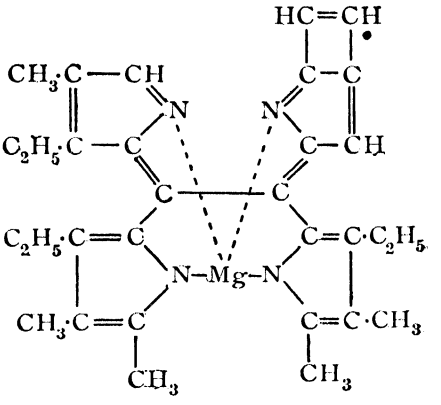


* Willstätter, *Ber.*, 1914, 47, 2854.

around which the methyl and ethyl groups are so disposed as satisfactorily to account for the products obtained on oxidation and reduction. In this way the following formulæ are provisionally given to ætioporphyrin and ætiophyllin:



Ætioporphyrin



Ætiophyllin

Carotin, $C_{40}H_{56}$, is an unsaturated hydrocarbon. It crystallizes in lustrous rhombohedra which are orange-red by transmitted and blue by reflected light. On exposure to the air it readily undergoes oxidation and becomes bleached. Carotin also occurs in the roots of carrot, and the colour of yellow or orange petals is not infrequently due to it.

Xanthophyll, $C_{40}H_{56}O_2$, forms yellow crystals with a blue lustre. Like carotin, it undergoes bleaching on exposure to the air. On reduction with magnesium powder and water xanthophyll is converted into carotin.*

Fucoxanthin, $C_{40}H_{54}O_6$, was first isolated from fresh brown algæ by Willstätter and Page.† Unlike carotin and xanthophyll, which are neutral substances, fucoxanthin has basic properties, and forms blue salts with mineral acids.

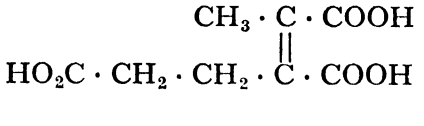
Chlorophyll and Hæmin. — Hæmoglobin contains 0·47 per cent of iron and consists of a colourless protein named globin, united to a coloured prosthetic or " non-protein " group. Hæmoglobin possesses the remarkable property of combining with carbon monoxide and nitric oxide, and is most easily oxidized to oxyhæmoglobin. When treated with weak acids, oxyhæmoglobin is easily resolved into globin and hæmatin. The nature of the union between these two substances is quite unknown.

* Ewart, *Proc. Roy. Soc.*, 1915, 89 [B], 1. † *Ann.*, 1914, **404**, 237.

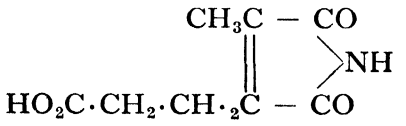
The properties of hæmatin have been studied by Kuster, Fischer (H.), Piloty, and Willstätter, but its constitution is still obscure. When treated with hydrochloric acid it is converted into a crystalline substance hæmin.

Both hæmatin and hæmin are freed from iron by the action of strong acids, and the iron-free pigment is known as hæmatoporphyrin. The latter on complete reduction with hydriodic acid yields hæmopyrrole. Willstätter and M. Fischer * treated hæmatoporphyrin with alcoholic potash in the presence of pyridine and obtained hæmoporphyrin, $C_{33}H_{36}O_4N_4$, which on distillation gave ætiophyllin. These observations are of special interest since they serve to co-relate chlorophyll and hæmin. Hæmoporphyrin is a dicarboxylic acid of ætioporphyrin.

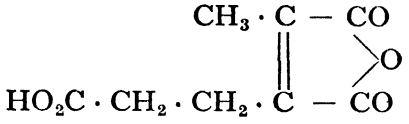
The oxidation products of hæmatin have been studied by Kuster.† Two hæmatinic acids, which eventually proved to be the anhydride and imide of carboxyethylmethylmaleic acid, were obtained:



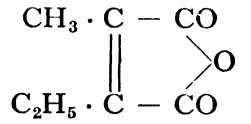
Carboxyethylmethylmaleic acid



Hæmatinic acid (i)



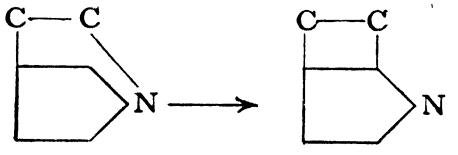
Hæmatinic Acid (ii)



Methylethylmaleic anhydride

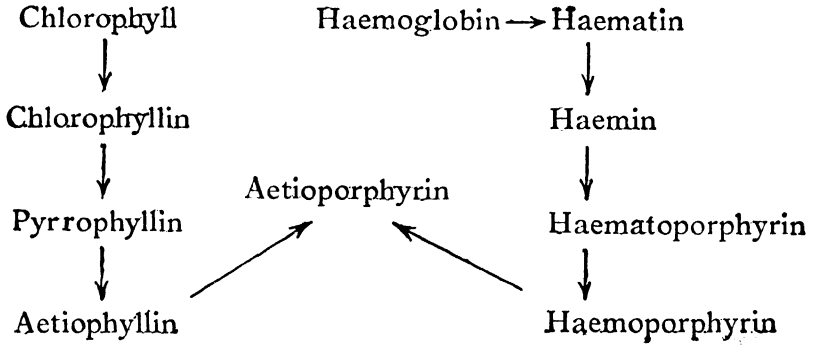
Both hæmatinic acids may be readily converted into methylethylmaleic anhydride.

It has not been found possible to eliminate the iron from hæmin until hydrogen bromide has been introduced into the molecule, and this is explained by a change from a nitrogen to a carbon linking:

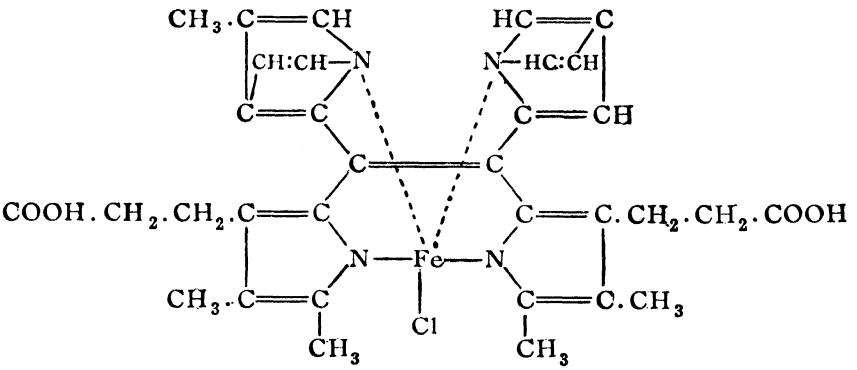


* *Zeit. Physiol. Chem.*, 1913, 87, 423. † *Ann.*, 1906, 345, 1.

The following tabulation briefly illustrates the relation of chlorophyll and its derivatives to those of hæmin:



The following provisional formula which has been assigned to hæmin should be compared with that of ætiophyllin:



Haemin

There are many facts which still require elucidation, and no doubt modifications in the above formulæ will be introduced as our knowledge of the more complex pyrrole derivatives becomes more complete.

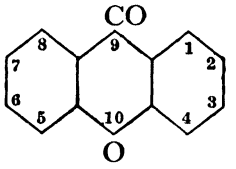
THE ANTHOXANTHINS

In this section we shall consider the more important yellow pigments which occur in the vegetative organs and in the petals of many plants. On account of their close relationship to the natural blue colouring matters known as the anthocyanins (p. 33), Willstätter and Everest* have proposed the adoption of the term "anthoxanthins" to distinguish these yellow pigments.

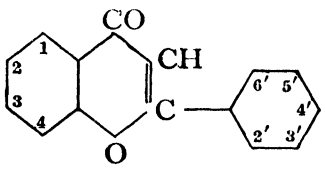
These colouring matters occur naturally in combination with

* *Ann.*, 1913 **401**, 189.

glucose or rhamnose, associated with the tannins, and in some cases uncombined. The majority of these pigments are derived either from xanthone (i) or flavone (ii), and are consequently frequently classified as the xanthones and flavones:



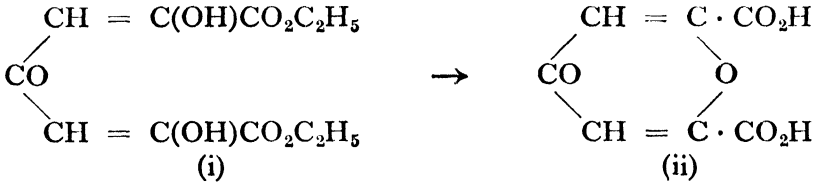
(i)



(ii)

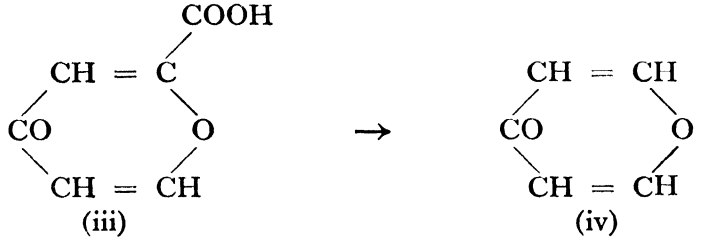
These compounds owe their chemical properties to the presence of the γ-pyrone nucleus, so that we may briefly consider the preparation of γ-pyrone, even although it is not itself a natural product.

γ-Pyrone.—This compound is the anhydride of a 1 : 5-dihydroxy-3-ketone, and has been prepared synthetically from chelidonic ester by Claissen * as follows: Chelidonic ester (i)† is converted into 2 : 6-pyrone carboxylic acid (ii) on treatment with fuming hydrochloric acid:



(i) (ii)

and on heating this substance decarboxylation takes place, with the successive formation of coumenic acid (iii) and γ-pyrone (iv):



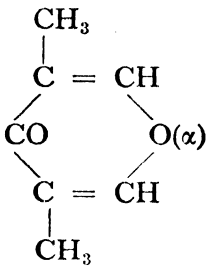
(iii) (iv)

γ-Pyrone is a colourless solid, and the basic properties of compounds containing the γ-pyrone nucleus are well marked. The salts with inorganic acids, which may be represented as addition products of the base with mineral acids, are as a rule more highly coloured than the bases from which they are derived, and are generally

* *Ber.*, 1891, **24**, 118.

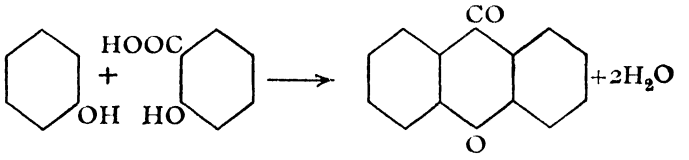
† Chelidonic acid (γ-pyrone dicarboxylic acid) occurs in combination with the alkaloid chelidonine in the root of the common celandine.

very unstable in the presence of water. In 1899 Collie and Tickle * observed that dimethyl γ-pyrone (v) readily forms salts with many acids, and they suggested that the existence of these salts is due to the oxygen atom (α) becoming quadrivalent when salt formation occurs.

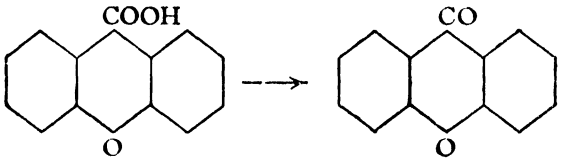


(v)

Xanthone was first obtained by Kolbe and Lautermann by the action of phosphorus oxychloride on sodium salicylate.† It is most conveniently prepared by distilling a mixture of acetic anhydride and salicylic acid. During this reaction some phenol is produced, and the reaction may be considered to consist of the condensation of phenol and salicylic acid:



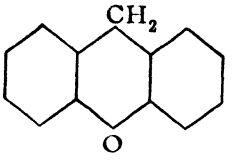
Ullmann and Zlokasoff ‡ obtained *o*-phenoxybenzoic acid (i) by the interaction of sodium phenate and sodium *o*-chlorobenzoate in the presence of copper powder, and this acid readily passes into xanthone by elimination of water.



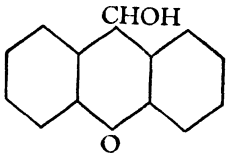
(i)

Xanthone crystallizes in long colourless needles, gives a blue fluorescence in sulphuric acid, and readily forms oxonium salts. By reduction under suitable conditions, xanthene (ii), xanthydrol (iii), and dioxyxanthylene (iv) may be obtained.

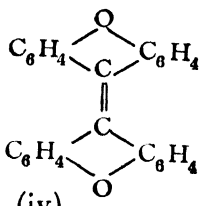
* *Trans.*, 1899, **75**, 710. † *Ann.*, 1860, **115**, 197.
‡ *Ber.*, 1905, **38**, 2111.



(ii)



(iii)

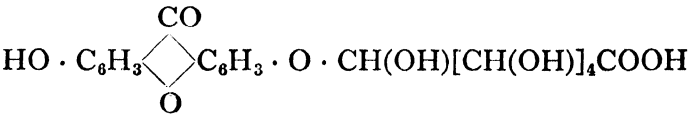


(iv)

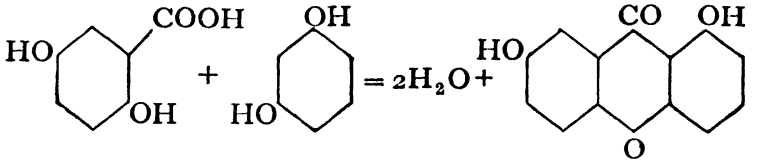
When the hydroxyxanthones, and indeed all hydroxyketones, are alkylated, the hydroxyl group in the ortho position to the carbonyl group remains unaffected. This is a noteworthy example of steric hindrance.

Indian Yellow or Piuri.—This pigment is obtained by concentrating the urine of cows which have been fed upon mango leaves. On account of its disagreeable smell it is seldom used as a dye-stuff.

The principal ingredient of this pigment is euxanthic acid ($C_{19}H_{18}O_{11}$), which is present in the form of its magnesium or calcium salt, for which Graebe * suggested the formula:

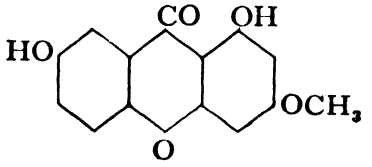


On treatment with dilute sulphuric acid, euxanthic acid yields euxanthone.† The latter has been synthesized by v. Kostanecki and Nessler ‡ by the condensation of resorcinol and hydroquinone carboxylic acid in the presence of acetic anhydride:



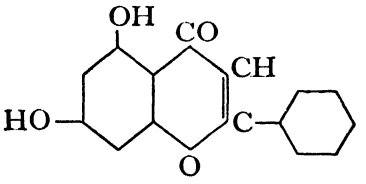
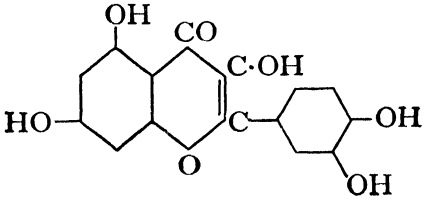
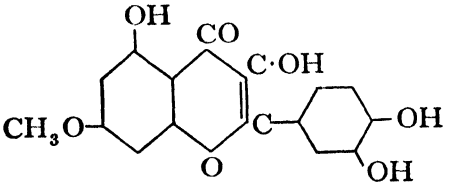
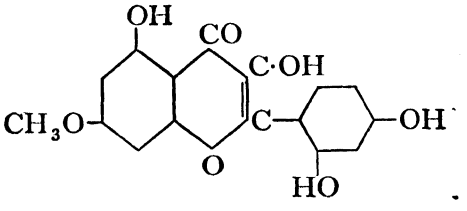
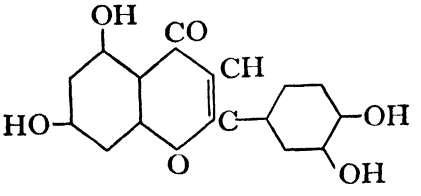
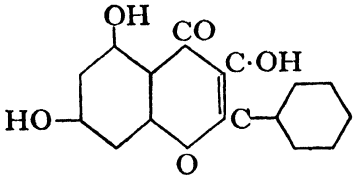
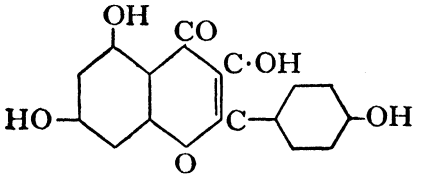
Euxanthone

Gentisin.—This pigment is the yellow colouring matter of *Gentiana lutea*, and its identity with 1 : 3 : 7-trihydroxyxanthone-3-methylether has been established by v. Kostanecki.§



* *Ann.*, 1889, **254**, 267.
† Cf. Stenhouse, *Ann.*, 1844, **51**, 429.
‡ *Ber.*, 1891, **24**, 1894.
§ *Monats.*, 1891, **12**, 207; 1895, **16**, 919.

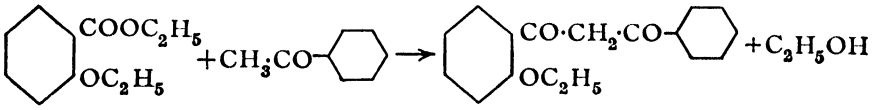
FLAVONE AND FLAVONAL PIGMENTS

Name.	Structural Formula.	Occurrence.
Chrysin		Several varieties of poplar, especially *Populus nigra*, *P. pyramidalis*.
Quercetin		Bark of *Quercus tinctorius*, leaves of horse chestnut, hop, &c.
Rhamnetin		Dried berries of *Rhamnus catharticus*, *R. tinctoria*.
Morin		Wood of *Morus tinctoria* (yellow wood).
Luteolin		*Reseda luteola*, "weld"; *Genista tinctoria*, "dyer's broom".
Galangin		Galanga root.
Kaempferol		Flowers of *Delphinium consolida*, *Prunus spinosa*; berries of *Rhamnus catharticus*.

The Flavones and Flavonols.—*Flavone* has been obtained in the free state in nature.* Many varieties of the primula possess on their flower stalks, leaves, and seed capsules a characteristic dust or meal. This powder, obtained from *P. pulverulenta* and *P. japonica*, has been shown to be identical with flavone.

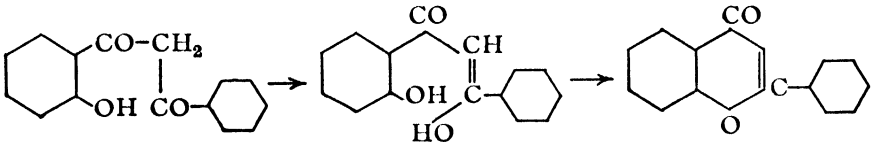
Flavone has been synthesized by several methods, of which one or two illustrations may be given:

1. Von Kostanecki and Tambor † condensed ethyl-*o*-ethoxybenzoate and acetophenone, in the presence of sodium, to give *o*-ethoxybenzoylacetophenone (i):



(i)

which, when digested with boiling hydriodic acid, yielded flavone.

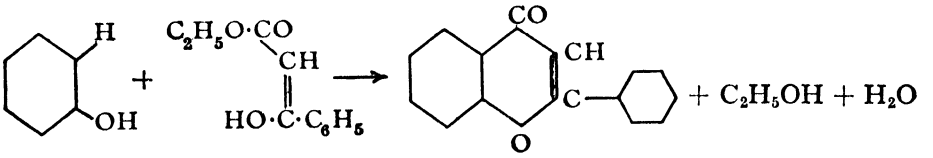


2. Ruhemann ‡ employed esters of β-hydroxyarylcinnamic acids, which he prepared by the action of sodium phenolates on the esters of propiolic acid, e.g.

$$C_6H_5C \vdots C \cdot CO_2C_2H_5 + NaOC_6H_5 = C_6H_5C(OC_6H_5) : C(Na)CO_2C_2H_5$$

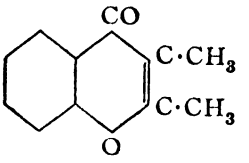
The esters were then converted into the corresponding acids and chlorides, and the latter on treatment with aluminium chloride gave the corresponding flavones:

3. Simonis § condensed benzoyl acetic ester and phenol in the presence of phosphorus pentoxide:



* *Trans.*, 1915, **107**, 872.
† *Ber.*, 1900, **33**, 330.
‡ *Ber.*, 1903, **36**, 1913, 2188.
§ *Ber.*, 1914, **47**, 2229.

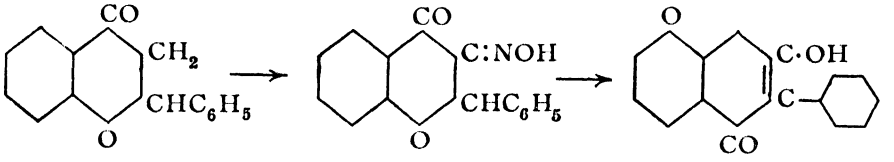
Methylacetoacetic ester condenses in the same way to give dimethyl-chromone:



It is interesting to note that if sulphuric acid is used in the condensation of phenols and β-ketonic esters, the reaction takes a different course and derivatives of α-pyrone (coumarin) are obtained.

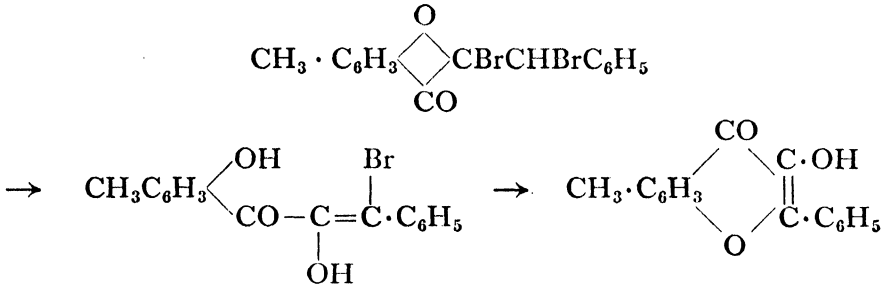
Flavonol differs from flavone in the fact that it contains a hydroxyl group in the γ-pyrone ring in the place of the hydrogen atom which is present in the case of flavone. Flavonol has been obtained synthetically as follows:

1. Von Kostanecki and Szabránski * converted flavanone into isonitroso flavanone (i) and thence into flavonol by the action of dilute acids:



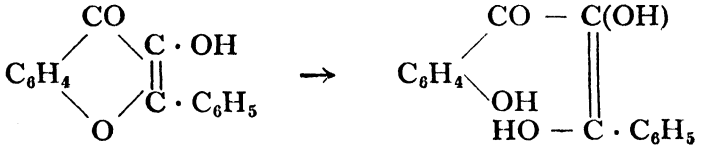
(i) Flavonal

2. Auwers and Müller † obtained 2-methyl flavonol by the action of caustic potash on the dibromide of benzylidene-4-methyl coumaranone:

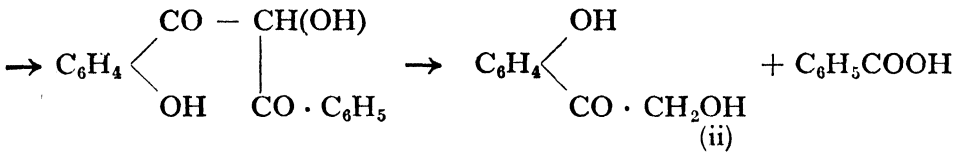


This is a general reaction and has been applied extensively.

On hydrolysis flavonol gives *o*-hydroxybenzoyl carbinol (ii) and benzoic acid:



* *Ber.*, 1904, 37, 2819. † *Ber.*, 1908, **41**, 4233.



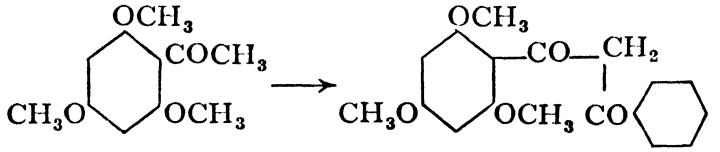
This reaction is typical of a whole series of flavonol derivatives, and has been generally applied in the determination of their structure. As a rule the compound is first fully methylated and then boiled with alcoholic potash.

Chrysin (1 : 3-dihydroxyflavone). — This pigment was first isolated from poplar buds, in which it is present to the extent of about 0·25 per cent, by Piccard in 1873. For this purpose an alcoholic extract of the buds is treated, while hot, with lead acetate, and after standing some time the yellow precipitate is removed. The excess of lead is removed as sulphide and the filtrate evaporated to dryness. The residue is then purified by recrystallization, after it has been successively extracted with carbon disulphide, benzene, and boiling water.

Chrysin crystallizes in colourless leaflets which in alkaline solution exhibit an intense yellow colour. It forms a diacetyl derivative. On boiling with concentrated potassium hydroxide solution, chrysin gives phloroglucinol, benzoic acid, acetic acid, and a little acetophenone:

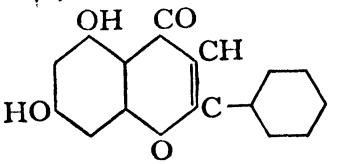
$$C_{15}H_{10}O_4 + 3H_2O = C_6H_6O_3 + C_6H_5COOH + CH_3COOH$$

The researches of v. Kostanecki indicated that chrysin was 1 : 3-dihydroxyflavone, and this has been established by its synthesis by Emilewicz, v. Kostanecki, and Tambor.* For this purpose phloroacetophenone-trimethyl-ether (i) is condensed with ethyl benzoate in the presence of sodium to give 2 : 4 : 6-trimethoxy-benzoyl-acetophenone (ii):



(i) (ii)

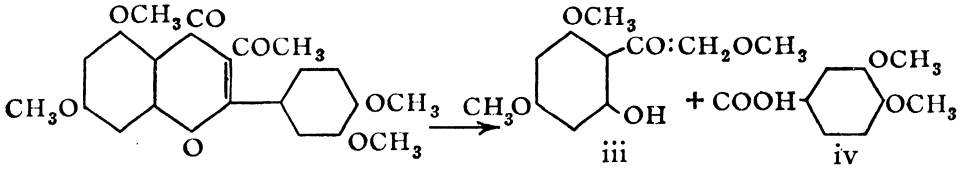
Boiling, concentrated hydriodic acid demethylates this compound, and at the same time the ring is closed with the production of chrysin:



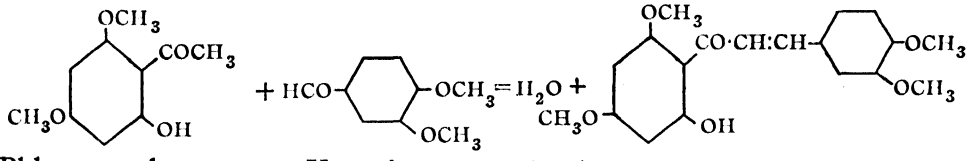
Chrysin

* *Ber.*, 1899, 32, 2448.

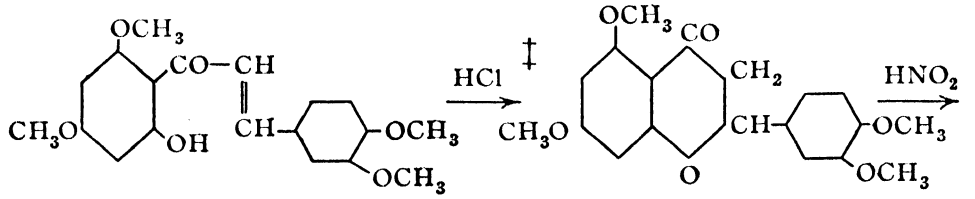
Quercetin (1:3:3′:4′-tetraoxyflavonol) has been the subject of several investigations. It gives a penta-acetyl derivative, both a mono- and a tetra-methyl ether, and when fused with alkali it yields protocatechuic acid and phloroglucinol. When pentamethyl quercetin is treated with alcoholic potash it gives methoxyfisetol-dimethyl-ether (iii) and veratric acid (iv), which indicates that quercetin is a derivative of flavonol: *

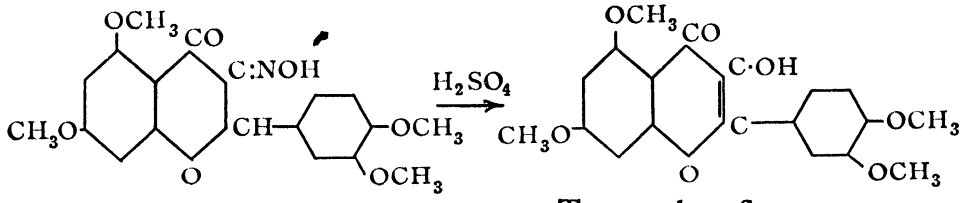


Quercetin has been synthesized from phloracetophenone dimethyl ether and veratric aldehyde by v. Kostanecki, Lampe, and Tambor as follows:†



Phloracetophenone dimethyl-ether | Veratric aldehyde | Hydroxytetramethoxy-benzal-acetophenone

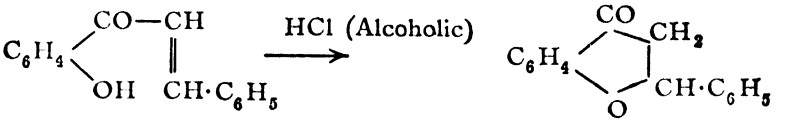




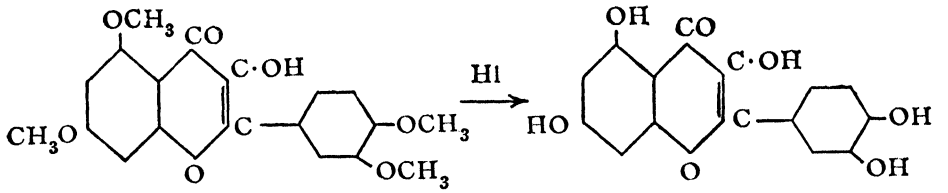
Tetramethoxyflavone

* Herzig, *Ber.*, 1909, **42**, 155. † *Ber.*, 1904, **37**, 1402.

‡ This is analogous to the reaction:



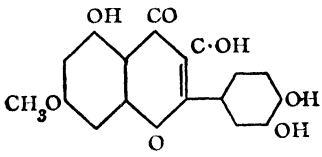
ω-Benzal-o-oxyacetophenone | Flavanone



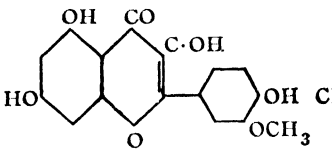
Quercetin

The reduction of quercetin to cyanidin chloride will be dealt with later (p. 37).

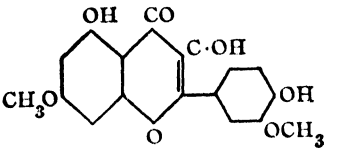
Rhamnetin (quercetin-3-methylether).—Persian Berries—the seed-bearing fruit of various species of Rhamnus—contain the glucoside xanthorhamnin, which on hydrolysis gives a sugar and the colouring matters rhamnetin, rhamnazin, and quercetin. Isorhamnetin, which is a monomethylether of quercetin, occurs, together with other products, in the common red clover.*



Rhamnetin



Isorhamnetin



Rhamnazin

Morin is obtained from " Old Fustic " the wood of *Chlorophora tinctoria*—a tree found in tropical regions. Together with logwood it is one of the most important natural dye-stuffs. In alkaline solution it gives an intense yellow colour, an olive-green coloration with ferric chloride, and a bright orange coloured precipitate with lead acetate.

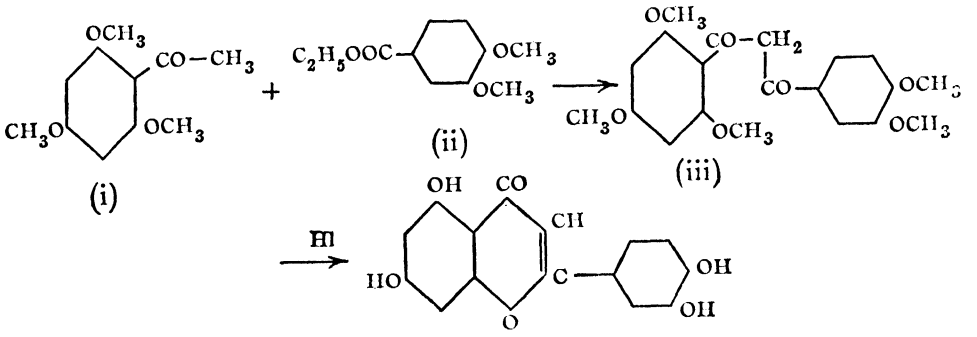
Morin has been synthesized by v. Kostanecki, Lampe, and Tambor in a similar manner to quercetin.†

Luteolin is the main colouring matter of " weld "—the dried herbaceous plant known as *Reseda luteola*—which is widely distributed in France, Germany, and Austria. Weld is probably the oldest European dye-stuff, and it was used by the Gauls in the time of Julius Cæsar.

A. G. Perkin assigned to luteolin the constitution of a tetrahydroxyflavone, and this structure has been confirmed by its synthesis by v. Kostanecki, Rozycki, and Tambor. For this purpose phloracetophenone trimethyl-ether (i) is condensed with ethyl-veratrate

* Power and Salway, *Trans.*, 1910, 97, 231. † *Ber.*, 1906, 39, 625.

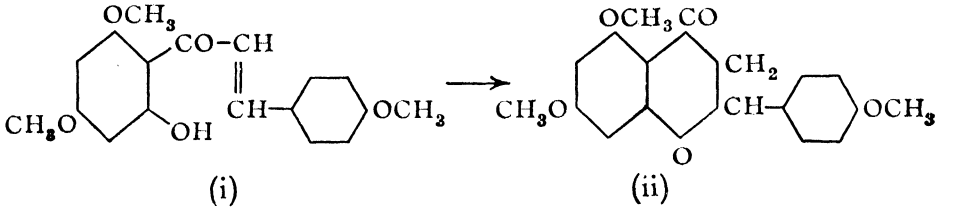
(ii) to give 2 : 4 : 6 : 3′ : 4′-pentamethoxy-benzoyl-acetophenone (iii), which on long digestion with concentrated hydriodic acid gives luteolin:



Galangin (dihydroxyflavonol) is obtained from galanga root —the rhizome of *Alpinia officinarum*. It has been obtained synthetically by v. Kostanecki and Tambor in a similar manner to that of Kaempferol.

Kaempferol.—The constitution of this compound as a trihydroxyflavonol is due to v. Kostanecki,* and it has been obtained from the blue flowers of *Delphinium consolida* by Perkin and Wilkinson.†

Von Kostanecki and Tambor ‡ have obtained kaempferol synthetically as follows: Hydroxy-4 : 6 : 4′-trimethoxy chalkone § (i), on boiling with alcoholic sulphuric acid, gives 1 : 3 : 4′-trimethoxyflavanone (ii):



The latter on treatment with amyl nitrite and hydrochloric acid yields isonitroso-1 : 3 : 4-trimethoxyflavanone (iii), which on boiling with 10-per-cent sulphuric acid gives 1 : 3 : 4′-trimethoxyflavonol

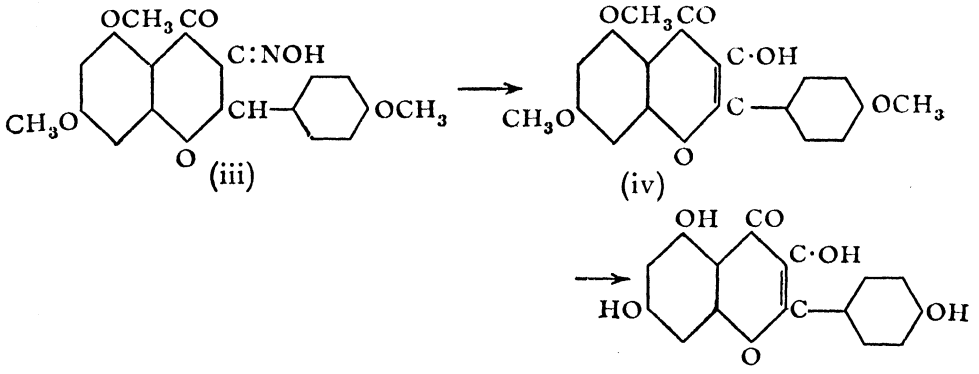
* *Ber.*, 1901, **34**, 3723. † *Trans.*, 1902, **81**, 585. ‡ *Ber.*, 1904, **37**, 792.

§ Chalkone or benzylidene acetophenone is readily prepared by the condensation of acetophenone and benzaldehyde:

$$C_6H_5COCH_3 + C_6H_5CHO = C_6H_5COCH_2CHOHC_6H_5$$
$$\rightarrow C_6H_5COCH:CHC_6H_5 + H_2O$$

(Claisen, *Ber.*, 1887, **20**, 257.)

(iv), and the latter on digestion with hydriodic acid yields kaempferol (v).



THE ANTHOCYANIN PIGMENTS

Introduction.—In spite of the fact that as early as 1664 Robert Boyle published an investigation of the colour changes which take place when flower extracts are treated with alkalies and acids, it is only within the last decade that the nature of the anthocyanins has been revealed.

The recognition of glucosides among the anthocyanins appears to have been first made by Heise as recently as 1894. In 1909 Miss Wheldale first suggested that anthocyanins might be formed from glucosides of the flavone or xanthone series by the action of oxidases, and showed that there are a certain number of anthocyanin types which give rise to a definite series of colour varieties.

Prior to 1913, the most fruitful attempt to isolate a colouring matter from blossoms in quantity sufficient for detailed examination had been made by Grafe in 1911, but the conclusions to which it led were inaccurate. In 1913 Willstätter began to publish, with numerous collaborators, a series of investigations which have brought this subject within the realm of synthetic chemistry. For the purpose of distinguishing the glucosidic from the non-glucosidic pigments, the names anthocyanin and anthocyanidin were applied to the former and the latter respectively.

The first of these papers was published in collaboration with Everest,* and dealt with the cornflower pigments. It was shown that the distinct shades of colour shown by different parts of the flower are due to various derivatives of one substance; thus the blue form is the potassium derivative of a violet compound which is

* *Ann.*, 1913, **401**, 189.

converted into the red form by oxonium salt formation with a mineral or plant acid. The chromogen, as found in blossoms, was combined with two molecular proportions of glucose and isolated as crystalline cyanine chloride. On hydrolysis, the sugar was removed and crystalline cyanidine chloride was obtained.

Continuing these investigations, it soon became evident that the almost infinite variety of colour and tint exhibited by flowers does not imply the existence of an equally diverse series of plant-colouring materials. It appears that only comparatively few basal colouring materials are distributed throughout the flower kingdom, and from these simple foundations the endless variety of floral shades and colours is built up by slight alterations in structure.

The colouring matter of a large number of flowers has been examined, and these investigations have now culminated in the synthesis of pelargonidin by Willstätter. In the early stages of the investigation of the anthocyanins, the reduction of quercetin was shown to produce cyanidin, and in this way the genetic relationship between the anthoxanthin and the anthocyanine series was established. More recent experiments on these lines have led to most interesting results concerning the problem of flower colorations.

Extraction of the Pigments.—In practice it has been found more advantageous to employ the dried material than to use fresh flowers. The solvents employed for the extraction vary according to the plant which is used as a starting material, but the essential part of the process is the formation of a sparingly soluble oxonium salt. Water alone suffices in the case of the cornflower; hydrochloric acid in methyl alcohol is used in the case of the rose and the hollyhock; while dilute alcohol is used to remove the pigments from the larkspur and the scarlet pelargonium. In the case of the grape the skins are extracted with glacial acetic acid at ordinary temperature and the dark red filtrate is precipitated with ether.

The investigation of the anthocyanins is rendered difficult by the fact that they are not very stable substances. Aqueous or alcoholic solutions of the pigments gradually lose their colour. Decolorization can be delayed by the addition of salts such as sodium chloride, and the addition of excess of mineral acid apparently stops it completely.

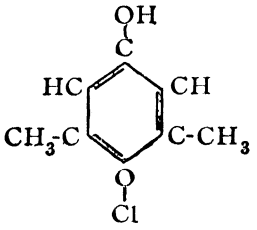
Among the methods which have been employed for the purification of the extracted pigments may be mentioned: precipitation and crystallization of the chloride; purification by suitable reagents and

crystallization of the chloride; and separation as picrate and subsequent conversion into chloride.

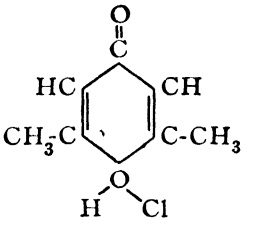
The anthocyanins can be distinguished from the anthocyanidins in solution by the addition of amyl alcohol after acidification with sulphuric acid, when the anthocyanidins alone dissolve in the amyl alcohol. The anthocyanins, as glucosides, are readily soluble in water, and as a rule in alcohol, but are insoluble in ether and chloroform. The glucosides are hydrolyzed by heating with dilute acids, and the resulting anthocyanidin salts are insoluble in ether but are generally soluble in water and in alcohol. With one exception, the pigments themselves have not been obtained in the crystalline state.

Nomenclature.—It has already been pointed out that when the anthocyanins are hydrolyzed by hydrochloric acid they are converted into sugars and the chlorides of a base—these chlorides being conveniently termed anthocyanidins.

The scientific terminology, however, requires some explanation. We have already seen that dimethyl γ-pyrone is a base which owes its basic properties to the fact that the oxygen atom may become quadrivalent with the formation of oxonium salts. These salts may be written (i) or (ii) according as we use the benzenoid or quinonoid formula:

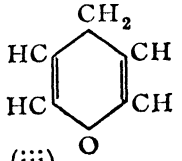


(i)

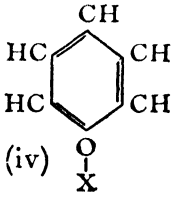


(ii)

The γ-pyrones may also be regarded as derivatives of γ-pyrone (iii), which again bears a resemblance to the pyrylium or pyroxonium salts (iv):



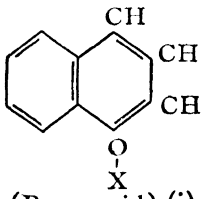
(iii)



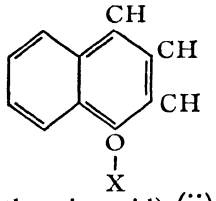
(iv)

Pyrylium salts such as the chloride and nitrate have been obtained, but the parent base has not been isolated.

The anthocyanidins may be regarded as derivatives of 2-phenyl-benzopyroxonium salts * which may be given a benzenoid (i) or a quinonoid (ii) structure. It is interesting to note that 2-phenyl-benzopyroxonium has not been isolated in a pure state. When its chloride, which itself is very hygroscopic and easily decomposed on exposure to the atmosphere, is treated with caustic soda, a very unstable crystalline precipitate is obtained.

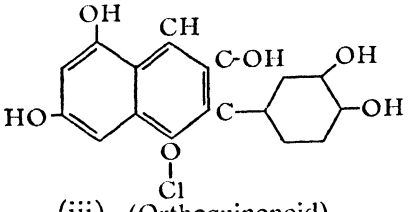


(Benzenoid) (i)

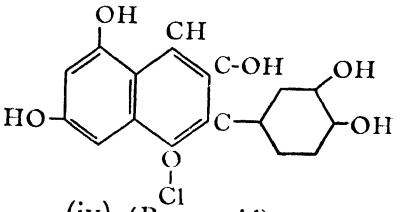


(Orthoquinonoid) (ii)

The anthocyanidins are hydroxy or methoxy derivatives of 2-phenyl-benzopyroxonium salts; thus cyanidin chloride may be written (iii) or (iv):

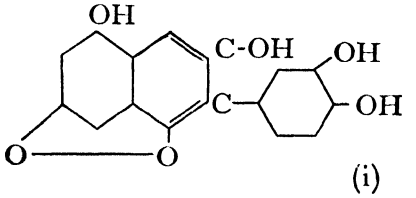


(iii) (Orthoquinonoid)



(iv) (Benzenoid)

It has been suggested that the blue anthocyanidin pigment is the potassium salt of a phenol betaïne (i), but this view has been adversely criticized by Heilbron.†



(i)

Distribution of the Anthocyanins.—The following tabulation illustrates the distribution of some of these pigments:

Pelargonidin.

Pelargonin.	Diglucoside of pelargonidin.	Dahlia, scarlet geranium.
Callistephin.	Monoglucoside of pelargonidin.	Aster.

* The salts of 2-phenyl-benzopyroxonium have been also termed polyflavylium salts or polyflavoxonium salts. In this way cyanidin chloride may also be designated as a pentahydroxy-flavoxonium chloride.

† Heilbron and Buck, *Trans.*, 1922, **121**, 1198.

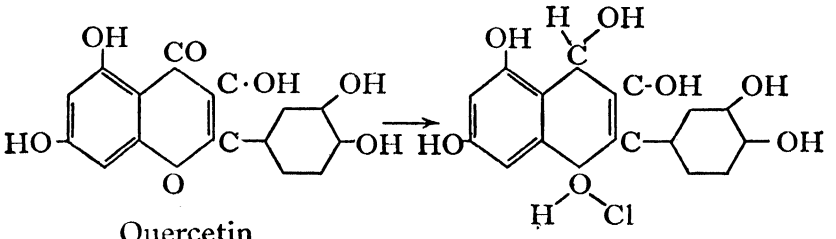
Cyanidin.

Cyanin.	Diglucoside of cyanidin.	Cornflower, dahlia.
Peonin.	Diglucoside of peonidin (monoethyl ether of cyanidin).	Peony.
Idaein.	Monogalactoside of cyanidin.	Fruit of cranberry.

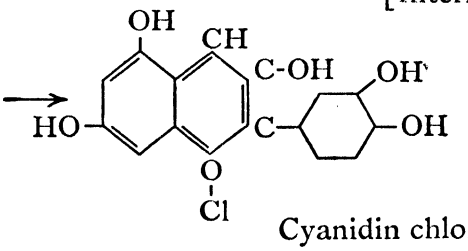
Delphinidin.

Violanin.	Rhamnoside of delphinidin.	Pansy.
Delphinin.	Diglucoside of delphinidin+*p*-hydroxybenzoic acid.	Larkspur.
Oenin.	Monoglucoside of oenidin (delphinidin dimethyl ether).	Fruit of grape.

Cyanidin Chloride.—Willstätter and Mallison obtained a small yield of cyanidin chloride by the reduction of quercetin with sodium amalgam or magnesium in alcoholic solution containing hydrochloric acid and mercury:



Quercetin [Intermediate product]

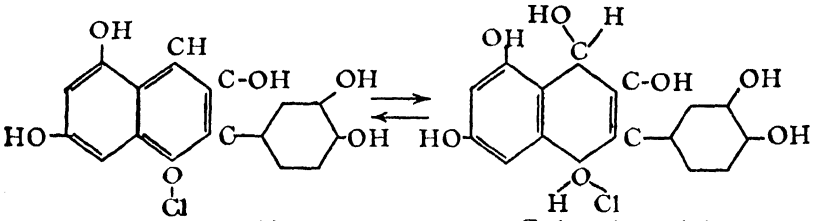


Cyanidin chloride

Cyanidin itself is insoluble in water, but readily soluble in alcohol. With sodium carbonate it resembles cyanin in first becoming blue and then violet. Colourless modifications of both cyanin and cyanidin are obtained by hydrolytic dissociation, and the colour may be restored by concentration or the addition of acid.

Everest found that cyanidin chloride undergoes decolorization when heated for a short time in dilute alcohol at 80°. In explanation of this phenomenon he suggests that in solution an equilibrium mixture of the two forms (i) and (ii) is present, and that preponderance of

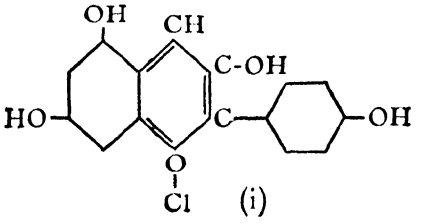
one or other of the two forms depends on the condition of the solution.



Coloured (i) Colourless (ii)

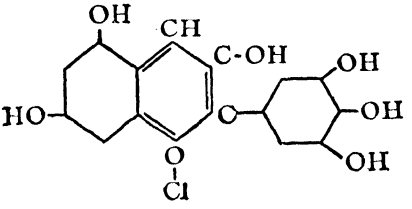
Pelargonidin and Delphinidin Chlorides.—Pelargonin is a monoglucoside of pelargonidin, and on hydrolysis with hydrochloric acid yields the chloride of the latter and glucose.

Phloroglucinol and *p*-hydroxybenzoic acid are obtained when pelargonidin chloride is heated with acids. This is in agreement with formula (i), and this structure has been confirmed by its synthesis.



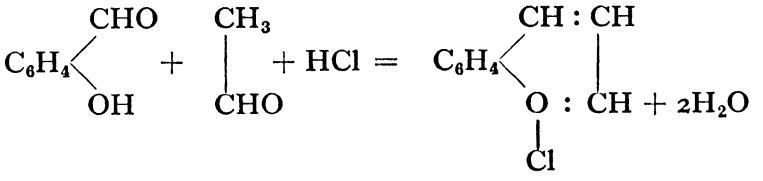
(i)

Delphinin chloride yields glucose, delphinidin chloride, and *p*-hydroxybenzoic acid on hydrolysis, and by analogy with the glucoside populin (benzoyl salicin) it is assumed that the benzoylation takes place in the glucose and not in the delphinidin portion of the molecule. Delphinidin chloride gives phloroglucinol and gallic acid on treatment with hot acids, and Willstätter has proposed the formula:

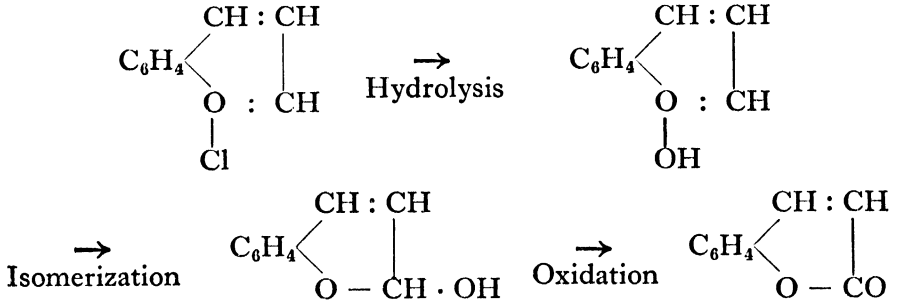


Synthesis of Salts of Benzopyroxonium and its Derivatives.—The following are the more important methods by which these compounds may be synthesized:

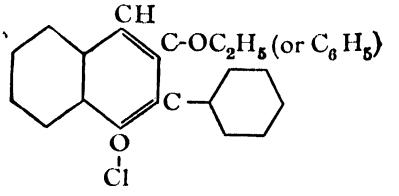
1. Decker and v. Fellenberg * obtained benzopyroxonium chloride by the condensation of salicylaldehyde with acetaldehyde in the presence of concentrated hydrochloric acid:



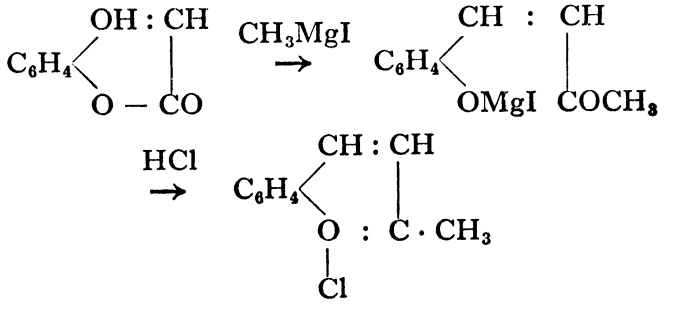
The salt was analysed in the form of its double salts with ferric chloride and gold chloride. In moist air the ferric chloride double salt undergoes slow decomposition with the formation of cumarin, probably according to the scheme:



When acetaldehyde is replaced by acetone, salts of 2-methyl-benzopyroxonium are obtained, and it has been generally shown that any compound containing the CH_2CO group may be used. In this way ω-ethoxy- and phenoxy-acetophenones have been converted into compounds of the anthocyanidin type.†



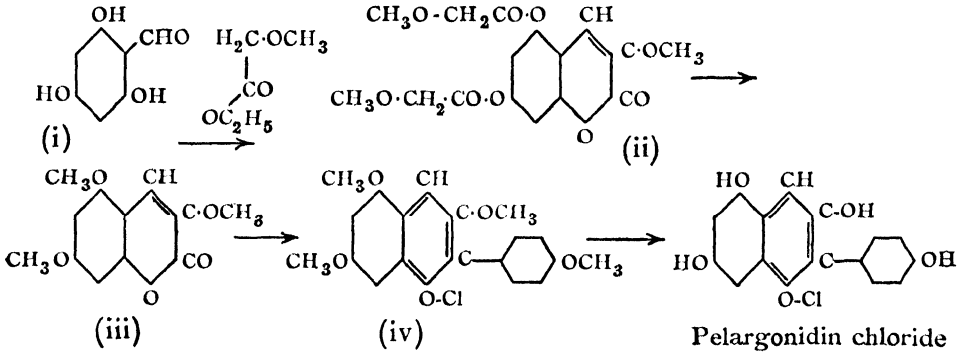
2. 2-Methyl-benzopyroxonium chloride has been obtained by treating cumarin with magnesium methyl iodide in benzol solution:



* *Ann.*, 1909, **364**, 17. † Pratt and Robinson, *Trans.*, 1922, **121**, 1577.

2-Phenyl-benzopyroxonium chloride has been obtained in a similar manner, using magnesium phenyl bromide.

Synthesis of Pelargonidin.—Pelargonidin has been synthesized by Willstätter and Zachmeister * as follows:—2 : 4 : 6-trihydroxybenzaldehyde (i) is condensed with sodium methoxyacetate, in the presence of the corresponding anhydride, to give 5 : 7-dimethoxyacetyl-3-methoxycoumarin (ii), which on successive treatment with caustic soda and diazomethane yields 3 : 5 : 7-trimethoxycoumarin (iii). This compound is then treated with magnesium anisyl bromide and converted into the chloride of anisyl-trimethoxybenzopyroxonium (iv). On demethylation with hydriodic acid and subsequent treatment with hydrochloric acid, pelargonidin chloride is obtained.



The Origin of the Anthocyanin Pigments in Plants.—This problem has attracted a good deal of attention, but up to the present no finality has been reached. In view of the frequent occurrence of glucosides in the plant it is not surprising that the anthocyanins are glucosides, but the non-glucoside or anthocyanidin portion of these compounds deserves special attention.

We have already seen that the anthocyanidins have a structural formula closely related to that of the flavones, and this fact in conjunction with the production of cyanidin on the reduction of quercetin has suggested that the anthocyanidins may be derived from the flavones.

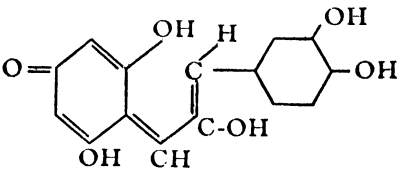
On comparing the formulæ of some of the anthocyanidins with those of the flavone and flavonol pigments, it is seen that they may be arranged in a series as follows:

Pelargonidin, $C_{15}H_{10}O_5$ . . . . Luteolin, kaempferol, fisetin, $C_{15}H_{10}O_6$
Cyanidin, $C_{15}H_{10}O_6$ . . . . Quercetin, $C_{15}H_{10}O_7$
Delphinidin, $C_{15}H_{10}O_7$ . . . . Myricetin, $C_{15}H_{11}O_8$

* *Sitz. Preuss. Akad. Wiss. Berlin*, 1914, **34**, 886.

From this tabulation it is evident that the anthocyanidins contain one atom of oxygen less than the corresponding flavones, so that if the chemist ascertains which flavone, flavonol, and anthocyanin pigments are present in one and the same flower, and then determines whether the relationship is one of oxidation or reduction, the problem will be advanced at least one stage.

Combes * has shown that if an acidified alcoholic solution of quercetin is treated with zinc dust, magnesium ribbon, or sodium amalgam, a brilliant crimson solution is obtained, which gives a green colour when treated with alkali. This red substance has been termed allocyanidin or " artificial anthocyanin ", but Willstätter has stated that it is not a true anthocyanin pigment and he proposes for it an open structural formula:



Shibata † and his collaborators have studied the reduction of myricetin by magnesium in the presence of organic acids, and have obtained a number of complex salts which appear to throw some light on the problem of plant coloration.

Despite the definite evidence produced by Willstätter and others, that anthocyanins are reduction products of flavones, the known correlation of distribution of oxydases and of anthocyanins may be used as an argument for the older oxidation hypothesis. It is quite possible, however, that the two views may be reconciled on the assumption that oxidation is needed in the earlier stages of the synthesis, and that only the final stage is one of reduction of flavone derivatives to anthocyanin.

Finally, it should be remembered that the fact that small quantities of a natural anthocyanin pigment can be obtained artificially by the reduction of a hydroxyflavonol, does not necessarily imply that one class is derived from the other in the plant.

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Untersuchungen über Chlorophyll, by Willstätter and Stoll (Berlin, 1913).

Ueber Pflanzenfarbstoffe, Willstätter (*Ber.*, 1914, **47**, 2831).

* *Comptes rendus*, 1914, **158**, 272. † *J. Amer. Chem. Soc.*, 1919, **41**, 208.

CHAPTER III

The Carbohydrates

Introduction.—This group of compounds, which includes the sugars, starches, celluloses, and gums, constitutes one of the most important groups of organic compounds. The carbohydrates are among the principal products of plant life, and they are also elaborated, but to a much smaller extent, in the animal organism.

The desire to produce grape-sugar artificially is coeval with organic chemistry itself, for Liebig had indicated the fascination of this problem. As early as 1811 Kirchhoff had found that starch may be transformed into a sugar, while more exact knowledge of the varieties of the sugars was obtained by Biot. The term glucose was suggested by Dumas, while lævorotatory fructose was termed lævulose by Berthelot. Kekulé eventually applied the term dextrose to dextrorotatory grape-sugar.

In 1861 Butlerow obtained methylenitan—by the action of lime water on a hot solution of trioxymethylene—as a sweet, pale yellow syrup responding to the common tests for glucose, but differing from the latter by being optically inactive and unfermentable by yeast. In 1886 Loew subjected formaldehyde to the action of cold lime water and obtained a sugar-like product which he termed formose. At this time chemists recognized two aldohexoses (glucose and galactose), two ketohexoses (fructose and sorbose), and one aldopentose (arabinose). Three hexobioses (sucrose, lactose, and maltose) and one hexotriose (raffinose) were also known to be definite individuals. Kiliani had introduced the cyanhydrin reaction, and had determined the general structure of glucose and galactose as that of straight-chained pentahydroxyaldehydes, and of fructose as a pentahydroxyketone.

The subsequent development of the sugars from this chaotic mass of isolated facts is largely due to the masterly researches of Emil Fischer. The discovery of plenylhydrazine was of enormous

value in this direction, and it was perhaps fortunate that nothing of the nature of the Walden inversion disturbed the aldohexose configurations. Step by step the structure of the monosaccharoses has been unravelled, and step by step they have been built up from the simplest materials.

Of late years the discovery of γ-methylglucoside by Fischer and by Irvine has opened the way to a multitude of contingent isomerides, those of *d*-glucose alone numbering eleven; and the work of Irvine on the substituted methyl derivatives of the carbohydrates has done much to increase our knowledge of their structure.

Even to-day our knowledge of the starches, celluloses, and gums is little more than a collection of empirical observations. The recent work of Irvine on the alkylation of these substances deserves particular commendation, because it is on these lines that the constitutions of many of these compounds may eventually be determined.

Classification of the Carbohydrates.—The carbohydrates fall naturally into two classes, the sweet and crystalline compounds termed sugars and the tasteless amorphous carbohydrates. According to the old system of classification, the carbohydrates were divided into three groups: the grape-sugar group, containing all the isomeric compounds of the formula $C_6H_{12}O_6$; the cane-sugar group, embracing all the compounds of the formula $C_{12}H_{22}O_{11}$; and the amylose or starch group, which contained the amorphous, complex carbohydrates of the general formula $(C_6H_{10}O_5)_n$. The three principal groups of carbohydrates are now termed **monosaccharoses** * (formerly glucose), **disaccharoses** (formerly the cane-sugar group), and **polysaccharoses** (formerly the starches, &c.).

Fischer succeeded in preparing a large number of new sugars, containing from two to nine carbon atoms, which possess the general characters of the monosaccharoses, and these are distinguished by the terms **biose, triose, tetrose,** &c. While some of the monosaccharoses combine the properties of alcohols and aldehydes, others have the characters of alcohols and ketones, and the additional terms **aldose** and **ketose** have been introduced. It should be noted that several recently discovered sugars, e.g. rhamnose and fucose, have the formula $C_6H_{12}O_5$, so that the old term "carbohydrate" (hydrate of carbon) is not now strictly applicable to all compounds classified as carbohydrates.

* Fischer uses the suffix "ide" in place of "ose", by analogy with the glucosides.

THE MONOSACCHAROSES

Natural Sources.—Glucose and fructose are among the most commonly occurring sugars in plants and animals. These and others may be obtained from the glucosides and polysaccharoses by the hydrolyzing action of acids and ferments. Thus *d*-glucose is found in the majority of glucosides, while cane-sugar yields glucose and fructose on hydrolysis, and raffinose yields glucose, fructose, and galactose.

Other monosaccharoses occur as follows:

ARABINOSE.—As the pentosan araban, in cherry gum, gum arabic, &c.

XYLOSE.—As the pentosan xylan, in woody tissue. It may be readily prepared from cotton seed hulls with a yield of 8 to 10 per cent.*

GALACTOSE.—As a galactan in various gums, mucilages, and pectans, e.g. agar-agar.

MANNOSE.—As condensation products, the mannans, in certain mucilages, and in the cell walls of the endosperm of various seeds. A convenient source for its preparation is vegetable ivory nut.†

Synthetic Preparation of the Monosaccharoses.—The following are among the more important synthetic methods which have been elaborated for the preparation of monosaccharoses:

1. **The Oxidation of the Polyhydric Alcohols.**—This may be effected by bromine and sodium carbonate,‡ nitric acid, Fenton's§ reagent (hydrogen peroxide and a ferrous salt), bromine on the lead salt of the alcohol,‖ platinum black.¶ E.g. glycerol gives glycerose, a mixture of glyceric aldehyde and dihydroxyacetone, in which the latter predominates.

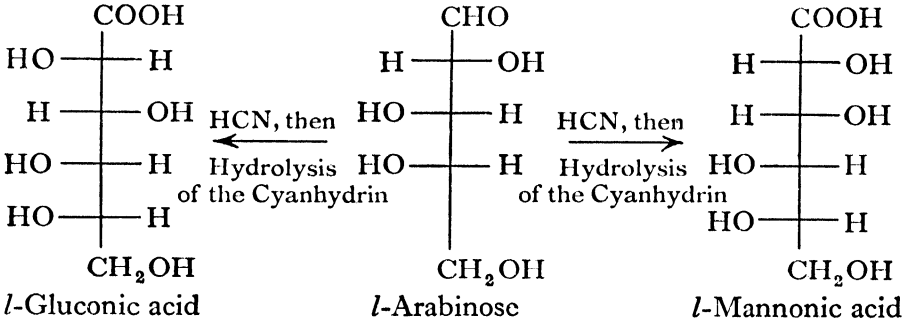
2. **Oxidizing Action by "Sorbose Bacterium".**—This organism was discovered by Bertrand** and found to exert a selective action in the case of the polyhydric alcohols. Glycerol and *i*-erythritol are oxidized to the corresponding ketoses, while glycol, *l*-xylitol, and dulcitol are unattacked.

* Hudson, *J. Ind. Eng. Chem.*, 1918, **10**, 176.
† Hudson, *J. Amer. Chem. Soc.*, 1917, **39**, 470.
‡ Fischer and Tafel, *Ber.*, 1887, **20**, 3384.
§ Fischer and Tafel, *Ber.*, 1887, **20**, 1088.
‖ Fischer and Tafel, *Ber.*, 1888, **21**, 2634.
¶ Grimaux, *Comptes rendus*, 1887, **104**, 1276.
** Bertrand, *Ann. Chim. Phys.*, 1904, 8, 3, 181.

3. **Aldol Condensation of the Lower Members in Alkaline Solution.**—Dilute sodium hydroxide solution is usually used as condensing agent, e.g. glycollic aldehyde polymerizes to erythrose:

$$CH_2(OH)CHO + CH_2(OH)CHO = CH_2(OH)\cdot CH(OH)\cdot CH(OH)\cdot CHO$$

4. **Conversion of a Lower to a Higher Monosaccharose. The Cyanhydrin Reaction.**—This reaction was discovered by Kiliani in 1885 *, and was widely applied by Fischer in the study of the sugar group. A typical example of its application is the conversion of *l*-arabinose into *l*-mannonic and *l*-gluconic acids:



A new asymmetric carbon atom is introduced in this reaction, and consequently two products are formed.

This reaction opened the way to the artificial production of *l*-glucose and *l*-mannose. The method was found to be capable of wide extension, and is limited only by the diminishing amount of material available for each succeeding step. The following series were realized by Fischer: †

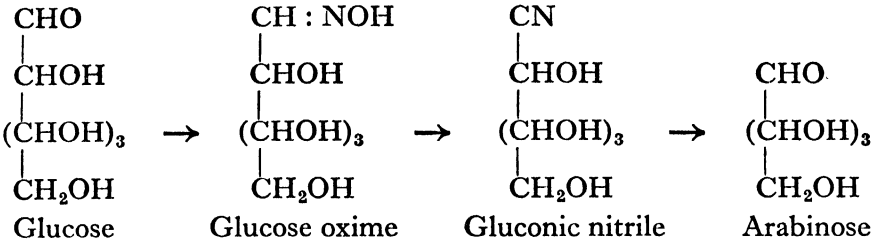
d-glucose →	{α-glucoheptose / β-glucoheptose} →	{α-gluco-octose / β-gluco-octose} →	glucononose
d-mannose →	mannoheptose →	manno-octose →	mannononose
$C_6H_{12}O_6$	$C_7H_{14}O_7$	$C_8H_{16}O_8$	$C_9H_{18}O_9$
rhamnose →	α-rhamnohexose →	rhamnoheptose →	rhamno-octose
$C_6H_{12}O_5$	$C_7H_{14}O_6$	$C_8H_{16}O_7$	$C_9H_{18}O_8$

5. **Conversion of a Higher to a Lower Monosaccharose.**—The degradation of a sugar may be brought about in four ways:

(*a*) *Wohl's method.*‡—This method may be illustrated by the conversion of glucose into *d*-arabinose. In practice the oxime of

* *Ber.*, 1886, **19**, 3033. † *Ber.*, 1890, **23**, 2611.
‡ *Ber.*, 1893, **26**, 730; 1897, **30**, 3101; 1899, **32**, 3666.

the monosaccharose is heated with acetic anhydride and a little zinc chloride, when a vigorous reaction ensues and the pentacetate of gluconic nitrile is formed. Hydrogen cyanide is then eliminated by treatment with ammoniacal silver oxide.



In this way glucose may be converted successively into arabinose, erythrose, glycerose, and glycollic aldehyde.

(*b*) *Ruff's method.**—In this method the calcium salt of the monobasic acid obtained from the higher monosaccharose is oxidized, by means of Fenton's reagent, to the lower sugar.

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \rightarrow \underset{\text{Gluconic acid}}{C_6H_{12}O_7} + O \rightarrow \underset{\text{Arabinose}}{C_5H_{10}O_5} + CO_2 + H_2O$$

(*c*) *Neuberg's electrolytic method.*†—The sugar is converted into the corresponding acid, and the copper salt electrolyzed between platinum electrodes. In this way gluconic acid may be converted into *d*-arabinose and ultimately complete degradation to formaldehyde achieved.

(*d*) *Weermann's method.*‡—An alcoholic solution of gluconolactone gives *d*-gluconamide on saturation with ammonia. When this is treated with hypochlorous acid a 50 per cent yield of *d*-arabinose is produced.

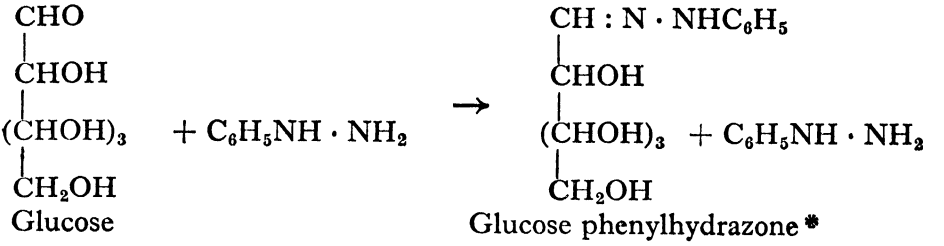
$$-CH(OH)CONH_2 \xrightarrow{HClO} -CH(OH)N:CO \xrightarrow{H_2O} -CHO + NH_3 + CO_2$$

In this way the following transformations have been achieved: *d*-galactose to *d*-lyxose, *l*-mannose to *l*-arabinose, and *l*-arabinose to *l*-erythrose.

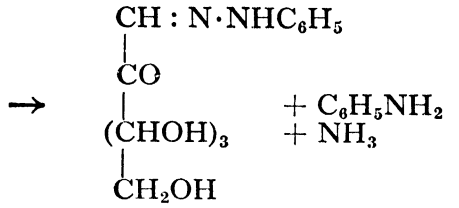
6. **Conversion of Aldoses into Ketoses.**—This change may be brought about with the aid of phenylhydrazine. The conversion of glucose into fructose as carried out by Fischer may be considered.

* *Ber.*, 1898, **31**, 1573. † *Biochem. Zeit.*, 1910, **24**, 152.
‡ *Absts.*, 1915, **1**, 387.

An excess of phenylhydrazine and glucose react with the formation of glucosazone as follows:

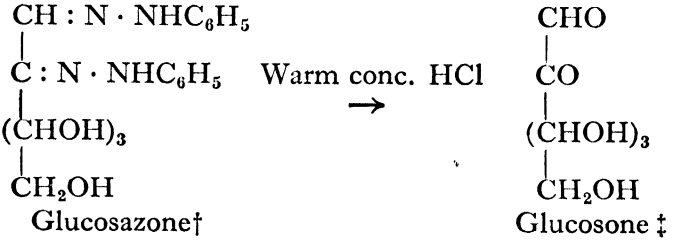


Glucose — Glucose phenylhydrazone*



Intermediate product.

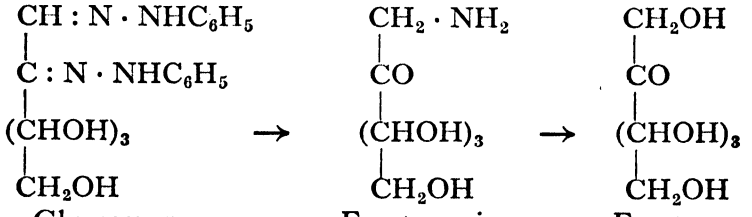
The phenylhydrazone first formed has undergone oxidation at the expense of a second molecule of phenylhydrazine, and the intermediate product thus formed then undergoes further condensation with phenylhydrazine to give glucosazone, which on hydrolysis gives glucosone.



Glucosazone† — Glucosone ‡

The osone on reduction of its lead salt with zinc dust and glacial acetic acid is converted into fructose.

An alternative method consists in reducing the osazone with zinc dust and acetic acid, when an osamine is formed. The latter on treatment with nitrous acid gives rise to fructose:



Glucosazone — Fructosamine — Fructose

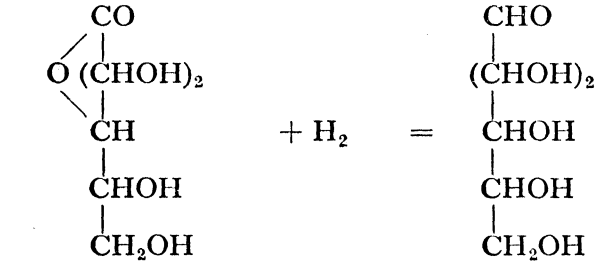
* Fischer, *Ber.*, 1887, **20**, 821.

† Fischer, *Ber.*, 1884, **17**, 579.

‡ Fischer, *Ber.*, 1889, **22**, 87.

It is interesting to note that Fischer has shown that the sugars may be conveniently purified by preparing the pure phenylhydrazone and regenerating the sugar therefrom by heating it with benzaldehyde or formaldehyde, when the hydrazone group is transferred to the aldehyde and oxygen takes its place.*

7. **Reduction of the Lactones of Mono- and Dibasic Polyhydroxy Acids.**†—The monocarboxylic acids, produced by the oxidation of the aldoses, are readily soluble in water, and many of them pass spontaneously into their lactones; e.g. gluconic acid gives gluconolactone. These lactones are crystalline compounds, which in aqueous solution pass into the corresponding acids, until a condition of equilibrium is attained. Many of these lactones may be reduced with sodium amalgam and water, in the presence of carbonic acid, and thus converted into the corresponding aldoses. The solution should be kept acidic by the judicious addition of dilute sulphuric acid from time to time as the sodium salt of the acid cannot be reduced. This is a reaction of great importance, since it serves as a means of passing from the acid to the corresponding aldose, which may then be reduced to the alcohol, e.g. gluconic lactone gives glucose:



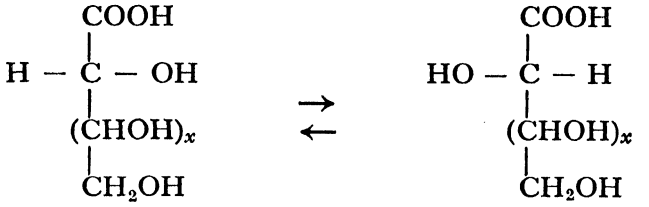
Gluconic lactone Glucose

8. **Interconversion of Isomeric Polyhydric Monocarboxylic Acids.**‡—These acids undergo a very interesting change when they are heated with pyridine or quinoline at a temperature of 130° to 150°, e.g. *d*-gluconic acid treated in this way is partly transformed into *d*-mannonic acid, whereas *d*-mannonic acid under the same conditions is partly converted into *d*-gluconic acid. The asymmetric carbon group, to which the carboxylic group is directly united, undergoes optical inversion. As the process is reversible the original and newly formed products are usually present is

* Fischer and E. F. Armstrong, *Ber.*, 1902, 35, 3141.

† Fischer, *Ber.*, 1890, 23, 930. ‡ Fischer, *ibid.*, 2611.

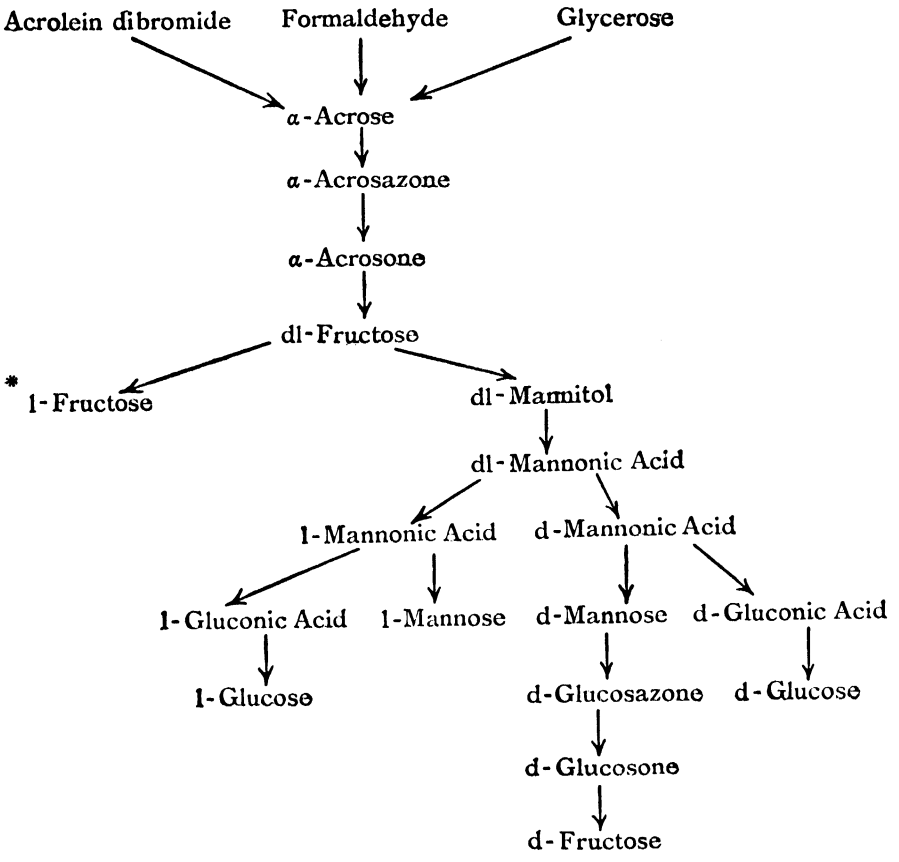
an equilibrium mixture. The reaction may be represented as follows:



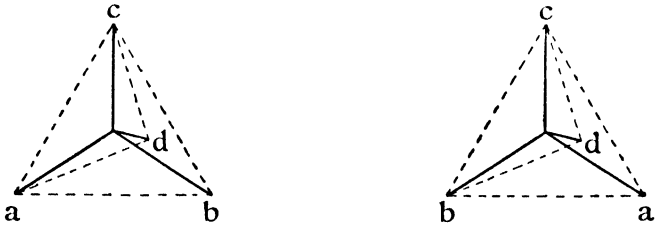
This method has proved of great value, not only for synthetic purposes, but also as a means of ascertaining the configuration of the monosaccharoses.

The Synthesis of Glucose and Fructose.—Although it is impossible to give here an adequate description of these syntheses, or to convey more than an idea of the great difficulties which had to be overcome, the more important results of this work may be briefly summarized.

The earlier work, carried out by Butlerow and Loew, has already been referred to. Fischer himself has stated that the directive influence on his work among the carbohydrates was the discovery of α- and β-acrose. In 1887, associated with Tafel, he obtained from acrolein dibromide and baryta a syrup which yielded two osazones, isomeric with one another and with phenylglucosazone. They were called α- and β-phenylacrosazone, which corresponded to the two synthetic sugars α- and β-acrose, having the composition $C_6H_{12}O_6$. The former sugar he subsequently identified with *dl*-fructose, whilst β-acrose, which he suggested resembled sorbose, was proved by E. Schmitz in 1913 to be the *dl* form of that ketose. The acroses are optically inactive, and although reducible to hexahydric alcohols the yields are very poor. β-Phenylacrosazone was eventually hydrolyzed to glucosone, and, on incomplete reduction, this product fructose was obtained. By applying this process to α-acrosazone, in combination at subsequent stages with Pasteur's methods of separating optical antipodes, the passage from inactive synthetic α-acrose to sugars identical in all respects with *d*-glucose, *d*-fructose, and *d*-mannose was ultimately effected. The following tabulated scheme illustrates the synthesis of some of these sugars.



The Configuration of the Monosaccharoses.—Before considering the stereochemical configurations of the monosaccharoses it is desirable to consider some simpler cases. Compounds of the type *Cabcd* which contain a single asymmetric carbon atom exist in two forms only. These may be represented thus:

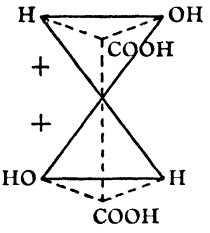


These two arrangements are related as object and mirror image. One may be designated as + or *d*, the other then becoming — or *l*,

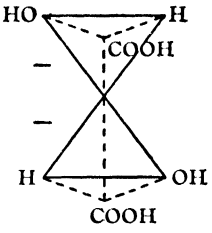
* As will be shown later, the formulæ assigned to *d*- and *l*-glucose are chosen arbitrarily. It is assumed that in the *d* form the groups occupy a certain position, so that in the stereoisomeride they are in the reverse position. The prefix does not necessarily denote the sense of the rotation.

the choice being immaterial. If these two compounds are solids the crystals of one are related to those of the other as object to mirror image. Such crystals are said to be hemihedral or enantiomorphous (Gr., ἐναντίος = opposite), and the *d* and *l* compounds are said to be enantiomorphously related to one another.

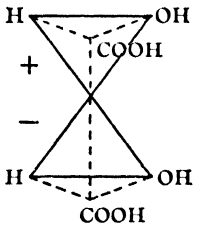
If two asymmetric carbon atoms are present a larger number of modifications may exist. The classic example of optical isomerism in substances containing two asymmetric carbon atoms is that of the tartaric acids. Four modifications, namely dextro, lævo, meso, and racemic, are known and the first three may be represented:



d-Tartaric Acid

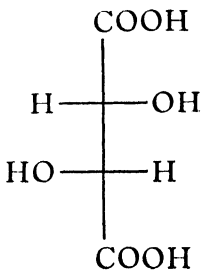


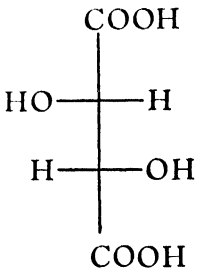
l-Tartaric Acid

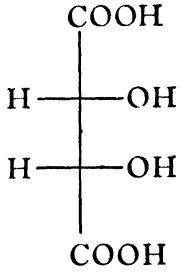


Meso-Tartaric Acid

or by using projections of these models:







Mesotartaric acid is the simple optically inactive form. It is inactive by internal compensation, and cannot be resolved into two optically active modifications because all the molecules of which it is composed are alike. Racemic or *dl*-tartaric acid is simply a crystallographic union of equal quantities of the *d* and *l* acids. It is therefore inactive by external compensation and can be resolved into the *d* and *l* forms.

Thus it is evident that compounds like tartaric acid, which contain two structurally *identical* asymmetric carbon groups, exist in three optically isomeric forms which may be represented by the signs − − , + + , and + − respectively. If, however, the two asymmetric groups in this acid are made to have *different* structures,

as e.g. by reducing *one* of the $-COOH$ groups to $-CH_2OH$, the configurations $+\ -$ and $-\ +$ are no longer identical, so that four optical isomerides are now possible, viz. $-\ -$, $+\ +$, $+\ -$, and $-\ +$. Of these four optical isomerides, the first two are enantiomorphously related, optically active forms. The second two are also enantiomorphously related and both are optically active. Neither would correspond with mesotartaric acid, because in the molecule of the latter the $+$ and $-$ groups are structurally identical and enantiomorphously related, as a result of which the acid is an internally compensated and optically inactive compound. The molecules of a tetrose, $CH_2OH \cdot CHOH \cdot CHOH \cdot CHO$, or the corresponding monocarboxylic acids, $CH_2OH \cdot CHOH \cdot CHOH \cdot COOH$, contain two *dissimilar* asymmetric carbon groups and would therefore exist in four optically active forms, and two of these ($-\ +$ and the $+\ -$) would give one and the same inactive dicarboxylic acid. An extension of this reasoning to the pentoses shows that the following tabulation represents the theoretical possibilities:

	Asymmetric Carbon Atoms.	Optical Isomerides.
Pentoses	3	8, 4 pairs of enantiomorphously related isomerides.
Corresponding monocarboxylic acid	3	8, 4 „ „
Corresponding dicarboxylic acids	3	4,* 4 „ „
Hexoses	4	16, 8 „ „
Corresponding dicarboxylic acids	4	10
Corresponding hexitols ..	4	10

Using the signs $+$ and $-$, as in the case of the tartaric acids,

* The molecules of a pentitol, $CH_2OH \cdot \overset{1}{C}HOH \cdot \overset{2}{C}HOH \cdot \overset{3}{C}HOH \cdot CH_2OH$, and those of the corresponding dicarboxylic acid, contain two asymmetric carbon groups only, because the middle atom is combined with two structurally identical groups [$-CHOH \cdot CH_2OH$ or $-CHOH \cdot COOH$], and has therefore lost its asymmetry. When the groups attached to 1 and 3 have the same configuration, $+$ and $+$ or $-$ and $-$, the compound is optically active (compare the tartaric acids). When these two asymmetric groups have different configurations (one being $+$ and the other $-$), internal compensation ensues and the presence of the middle CHOH group (2) renders possible the existence of two such internally compensated (inactive) forms. There are thus four optically isomeric pentitols, of which two are enantiomorphously related and optically active, and two are inactive by internal compensation.

to distinguish any two enantiomorphously related groups, the configurations of the aldopentoses and aldohexoses may be represented thus:

Aldopentoses.

	1	2	3	4	5	6	7	8
CH_2OH								
(X) CHOH	+	+	−	−	+	+	−	−
* CHOH	+	−	+	−	+	−	+	−
(Y) CHOH	−	−	+	+	+	+	−	−
CHO								

Aldohexoses.

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
CH_2OH																
CHOH	+	+	+	+	−	−	−	−	+	+	+	+	−	−	−	−
CHOH	+	+	−	−	+	+	−	−	+	+	−	−	+	+	−	−
CHOH	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−
(β) CHOH	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
CHO																

The Pentoses and Hexoses.—Lævoxylose under suitable treatment gives one of the optically inactive pentitols and one of the optically inactive trihydroxy-dicarboxylic acids, and therefore must be represented by 1, 2, 3, or 4, since these are the only configurations from which it would be possible to derive an internally compensated pentitol, i.e. a pentitol in which the two asymmetric carbon groups (X) and (Y) are of different signs. Lævoarabinose, on the other hand, gives one of the optically active pentitols and therefore its configuration must be either 5, 6, 7, or 8.

Lævoarabinose forms a cyanhydrin, which on hydrolysis gives the structurally identical but optically isomeric *l*-gluconic and mannonic acids. On reduction of the lactones of these acids, *l*-glucose and *l*-mannose are formed respectively. In these reactions the new asymmetric carbon atom β is introduced. The configurations of *l*-glucose and *l*-mannose must therefore be included in the series IX to XVI.

* See p. 52.

Lævoglucose and *l*-mannose give optically active dicarboxylic acids and optically active hexitols. This excludes XII or XIII, because the dicarboxylic acids and hexitols derived from these would be optically inactive by internal compensation. (In the compounds COOH + − + − COOH or CH_2OH + − + − CH_2OH, the asymmetric groups in corresponding positions, are enantiomorphously related and cause internal compensation.) Therefore *l*-glucose and *l*-mannose must be IX, X, XI, XIV, XV, or XVI.

Dextroglucose and *d*-mannose give one and the same osazone. Therefore the molecules differ in configuration only as regards that particular asymmetric group directly united to the aldehyde group (*β*) (p. 53). This excludes structures XI and XIV, since on excluding the *β* group the other three asymmetric groups are not identical. The configurations must therefore be chosen from IX, X, XV, or XVI.

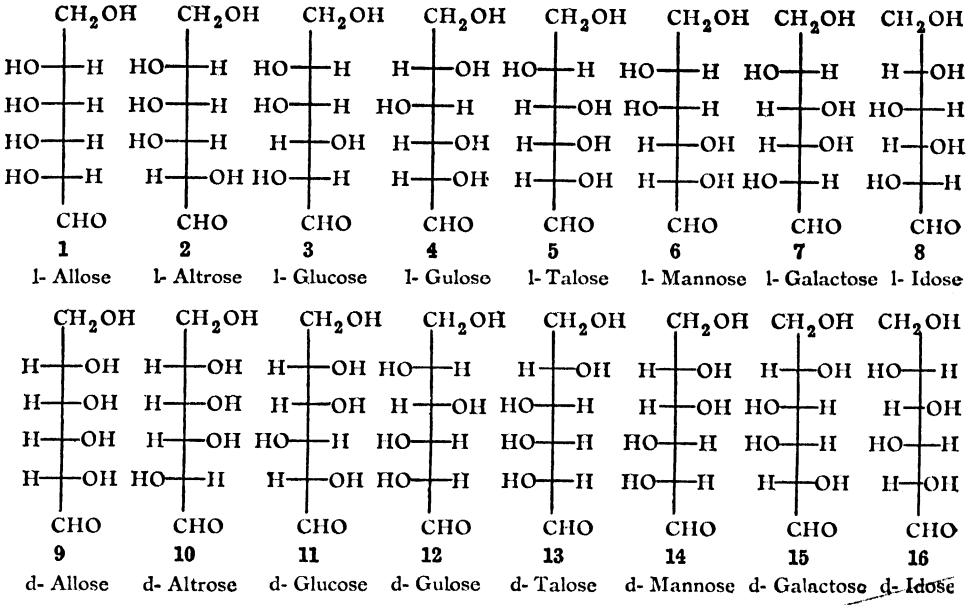
Dextroglucose and *d*-gulose give on oxidation one and the same optically active dicarboxylic acid (*d*-saccharic acid), and on reduction one and the same optically active hexitol (*d*-sorbitol). This excludes IX or XVI, because a dicarboxylic acid or hexitol having the corresponding configurations could not be produced from two different aldohexoses. Lævoglucose must therefore be either X or XV.

Since the signs + and − were chosen quite arbitrarily and the same relationship would hold if all the signs had been reversed, *one* of these configurations may be arbitrarily assigned to *d*-glucose, the other to *l*-glucose.

If XV is assigned to *l*-glucose, *d*-glucose becomes X, *d*-mannose IX, *l*-mannose XVI, *d*-gulose V, *l*-gulose IV, while *l*-arabinose, from which *l*-glucose and *l*-mannose are obtained, becomes 8, *d*-arabinose 5, and *l*-xylose (from which *l*-gulose is derived) 2.

The optical relationships of the various members of the hexose group are shown more fully by means of projection formulæ* in the tabulation on p. 55. As already mentioned, the choice between the letters *d* and *l* has been made by Fischer to depend on the structural relationship of these compounds rather than on the direction in which the substances rotate the plane of polarized light (p. 50).

* Cf. Tartaric Acids, p. 51.



Configurations of the Aldohexoses.

Enzymes.—Before discussing the fermentation of the monosaccharoses, it would be advisable to briefly consider the enzymes (ἐνζύμη = in yeast)—the lifeless products of the living cells which directly induce these changes and act either in the presence or absence of the living organism.

Enzymes are substances of the utmost importance to all living matter, and they may be regarded as the "chemical reagents" of the organism. It has not been possible as yet to isolate an enzyme in the pure state, and up to the present it has been impracticable to do more than investigate the effects produced when mixtures containing them are allowed to act upon substances of known composition. In many cases it is possible to obtain solid amorphous preparations which furnish extremely active enzyme solutions when dissolved in water. Unfortunately there is no definite criterion of purity for these colloids, and the methods available for their preparation are such as would not remove many known impurities.

As a rule enzymes are thrown out of solution on addition of alcohol or salts such as ammonium and sodium sulphates, while they are frequently carried down with neutral precipitates such as calcium phosphate, when formed in their presence.

Willstätter and Stoll* have endeavoured to improve the methods

* *Ann.*, 1918, **416**, 21.

for isolating and purifying enzymes so that the following points may be settled: (1) whether enzyme activity is possessed by an analytically pure compound or whether an enzyme is a system of co-operating substances; (2) whether a metal is an integral part of an enzyme; and eventually (3) what atomic groupings are associated with enzyme activity. It is too early as yet to answer any of these questions definitely. As a preliminary study the case of horse-radish peroxydase was chosen. Still more recently the same authors * have devised an improved method of purification by means of adsorption compounds with aluminium or ferric hydroxide, silicic acid, kaolin, or talc. Similar methods have been applied to invertase.†

Enzymes can act only within a limited range of temperature. In general the enzymes of animal origin act best at 37°, while 25° is a suitable temperature for those of vegetable origin. With few exceptions enzymes can act only in neutral solution, and a faintly acid medium is preferable to an alkaline one. Many neutral substances which are very poisonous to living organisms are not nearly so prejudicial to the enzymes, so that substances of this kind are frequently added to solutions in which enzyme changes are in progress in order to prevent bacterial contamination.

Yeast juice containing zymase is a complicated mixture of enzymes. The process of dialysis serves to separate it into two parts, the dialysate, which has passed through the membrane, and the residue which has not. Each of these portions by itself is incapable of effective fermentation, but when mixed together they become active. The active substance contained in the dialysate is called the *co-enzyme*.

Fermentation of the Monosaccharoses.—During the course of his experiments on racemic acid, Pasteur observed that aqueous solutions of the acid become lævorotatory in the presence of *penicillium*, owing to the destruction of the dextrotartaric acid by the fungus, and this device has been frequently employed for the resolution of inactive mixtures. Fischer has shown that this selective action is exhibited by invertase and zymase in producing fermentation of carbohydrates. Of the aldohexoses only the three natural sugars, *d*-glucose, *d*-mannose, and *d*-galactose are fermentable. The tetroses, pentoses, heptoses, and octoses are not attacked by yeast, while mannononose undergoes alcoholic fermentation and glycerose

* *Ann.*, 1921, **422**, 47. † *Ann.*, 1921, **425**, 1.

is partly transformed into propionic acid. It would seem, therefore, that the only molecules which are attacked are those which contain three, or a multiple of three, carbon atoms.

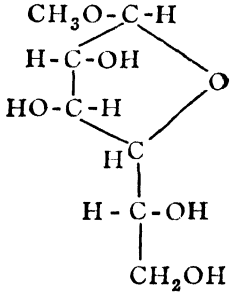
It is obvious that the action of the enzyme depends not only on the structure but also on the configuration of the molecule, and to explain this selective action Fischer introduced the simile of a "lock and key". When the asymmetric structure of the enzyme corresponds to that of the organic compound to be acted upon, or "the wards of the key fit those of the lock", reaction may occur.

The selective action of Bertrand's sorbose bacterium has already been mentioned.

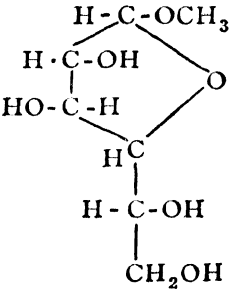
The Methylglucosides.—In 1893 Fischer observed that when a solution of glucose in cold methylalcohol was saturated with dry hydrochloric acid and allowed to stand, the mixture lost its characteristic aldehydic property of reducing cupric solutions. On concentrating the solution, after neutralization with lead carbonate, crystals of α-methyl glucoside separated while the mother liquors contained the isomeric β-compound.

	Melting Point.	Rotatory Power.
α-Methyl glucoside ..	165°	+ 157°
β-Methyl glucoside ..	104°	— 33°

The two products are regarded as stereoisomeric γ or butylene oxides, and have the following structural formulæ:



α - Methyl Glucoside



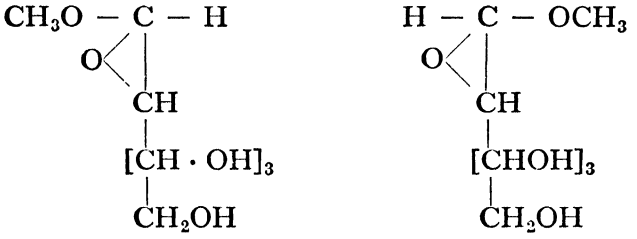
β - Methyl Glucoside

This type of ring is often termed the pentaphane or butylene oxide ring. When hydrolyzed by acids these glucosides yield methyl alcohol and glucose. The action of enzymes towards them is specific, for each form requires its own particular enzyme; thus, α-methyl glucoside is hydrolyzed by maltase and β-methyl glucoside by emulsin.

In 1914, Fischer isolated a third product from this reaction in the form of a syrup. He found that the syrup could be distilled in a high vacuum, and had the composition of a methyl glucoside ($C_7H_{14}O_6$). This isomeric methyl glucoside is scarcely attacked by emulsin or maltase. Irvine, Fyfe, and Hogg * have shown that this compound is a mixture of isomerides derived from an entirely new variety of glucose. It readily condenses with acetone, reduces alkaline potassium permanganate, and unites with oxygen to give a neutral product. When methylated by the combined action of silver oxide and methyliodide, it gives a new tetramethyl-methylglucoside which, on hydrolysis, is converted into a liquid tetramethylglucose. This compound is very reactive but forms no phenylosazone. On reduction it gives a tetramethylhexitol which is probably correctly represented by the formula:

$$CH_2(OCH_3) \cdot [CH \cdot OCH_3]_3 \cdot CHOH \cdot CH_2OH.$$

On this basis an ethylene oxide structure must be assigned to the new tetramethylglucose, and the new methylglucoside is a mixture of stereoisomerides having the formulæ:

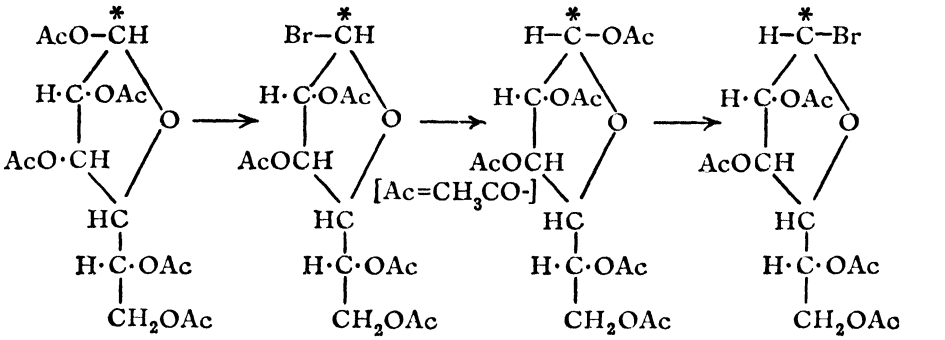


The parent glucose has not yet been isolated in the free state.

In addition to α- and β-methyl glucosides, there are many other derivatives of α- and β-glucose. Under proper experimental conditions all five hydroxyl groups in glucose become acetylated, the α- or β-pentacetate predominating according to the method adopted.

In either isomeride, one of the acetyl groups—that attached to the carbon atom marked with an asterisk—is far more reactive than the rest. When either of these compounds is treated with liquid hydrogen chloride or bromide, or, more easily, by the action of a saturated solution of these two acids in acetic acid, it is converted into the corresponding α- or β-acetochloro- or acetobromo-glucose:

* *Trans.*, 1915, **107**, 524.



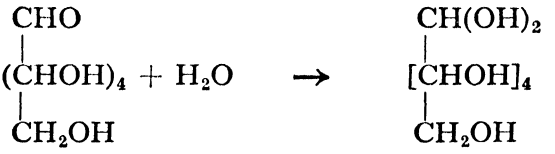
α Glucose pentacetate α Acetobromoglucose β Glucose pentacetate β Acetobromoglucose

Fischer and his collaborators have utilized acetobromoglucose and similar derivatives of other hexoses for obtaining a variety of glucosides.

In addition to the isomeric forms of glucose pentacetate, acetochloro- and acetobromo-glucose, the following glucose derivatives are known: α- and β-acetonitroglucose, α- and β-acetomethylglucoside, α- and β-tetracetylglucose, and various methylglucoses and methylglucosides.

Mutarotation: the Isomeric Forms of Glucose.—The gradual fall of optical rotation observed when a freshly prepared solution of glucose is allowed to stand is known as mutarotation. The change takes place very slowly when highly purified glucose is used, but almost immediately if a small quantity of alkali is added.

In 1890, Fischer noticed that certain lactones related to the sugars underwent a similar change, and he therefore ascribed the change with glucose to a like addition of a molecule of water, with the formation of a heptahydric alcohol.



In 1895, Tanret * described a further form of glucose of constant rotatory power, and three forms of glucose were now recognized:

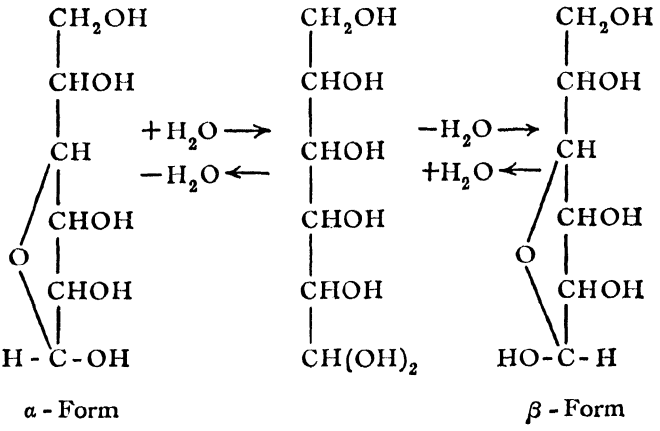
α-glucose, $[\alpha]_D$ + 110° to + 52·5°
β-glucose, $[\alpha]_D$ + 19° to + 52·5°
γ-glucose, $[\alpha]_D$ + 52·5°

Simon † compared the optical behaviour of α- and β-glucose

* *C. r.*, 1895, **120**, 1060. † *C. r.*, 1901, **132**, 487.

with the corresponding methylglucosides and suggested that both contain a closed ring. Direct proof of the glucosidic structure of both α- and β-glucose was afforded by their preparation from the corresponding glucosides by Armstrong,* who used appropriate enzymes for the hydrolysis.

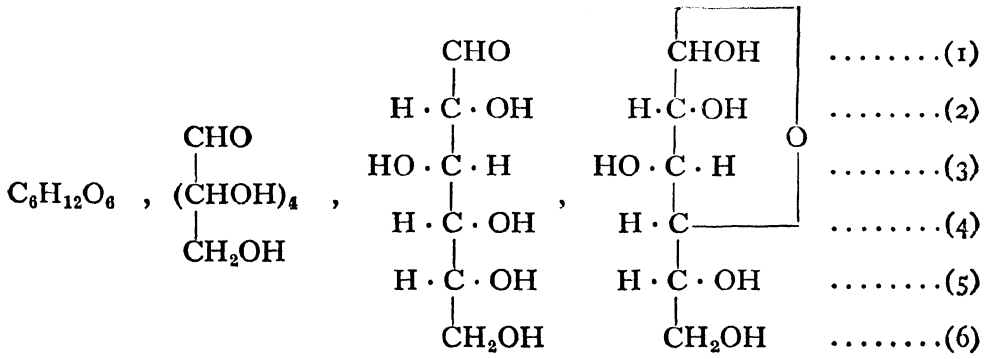
The change in rotatory power of glucose was shown by Lowry in 1899, to be a process of reversible isomeric change and he subsequently concluded that Tanret's γ-glucose is a mixture in which α- and β-glucose are present in equilibrium. Lowry† assumes that an aldehyde or hydrate is an intermediate stage in the establishment of equilibrium between the glucoses:



α-Form β-Form

This explanation involves the opening of the ring, and an alternative formulation has been put forward by Armstrong which does not involve any disruption of the γ-oxide ring.

The stages through which our present views regarding the glucose molecule have developed may be expressed in historical sequence as follows:



* *Trans.*, 1903, 85, 1306. † *Trans.*, 1903, 85, 1314.

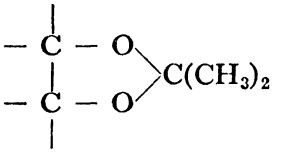
Position 1 in the last formula plays a part in mutarotation, in the formation of glucosides, and in oxidation processes, while the properties of the group indexed as 2 are revealed in the formation of osazones. Our knowledge of the group in position 6 is restricted to a few reactions such as oxidation to saccharic acid and the formation of dibromoderivatives. It is evident that the linkage of the ring-forming oxygen atom in the molecule need not remain exclusively in one position, but may connect different pairs of carbon atoms, and thus all the groups from 1 to 6 must be regarded as variables. For example:

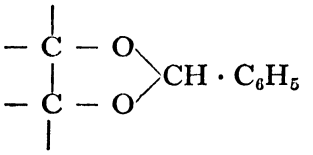
Position 1 may possess either the α or β configuration.
„ 2 may be involved in an ethylene-oxide ring.
„ 3 „ „ a propylene-oxide ring.
„ 4 „ „ a butylene-oxide ring.
„ 5 „ „ an amylene-oxide ring.
„ 6 „ „ a hexylene-oxide ring.

It follows, therefore, that if we include an aldehydic variety, *d*-glucose may react in any one of eleven forms or as a mixture of these isomerides.

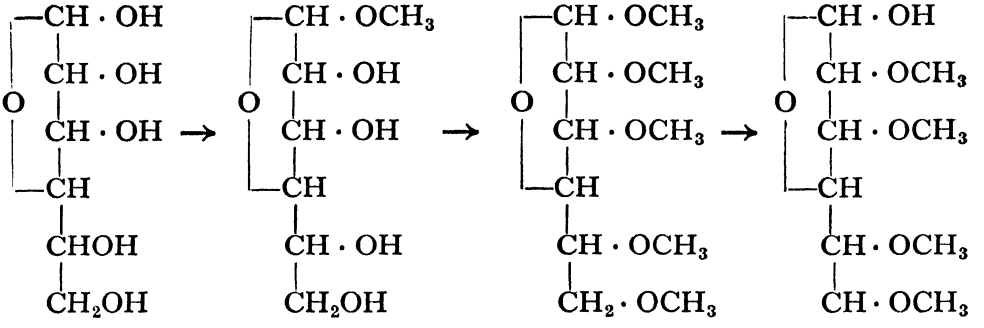
Methylated Sugars. — The reactive properties of the hydroxyl groups in glucose can be masked by acetyl or benzoyl groups, but these groups are too easily removed in subsequent reactions, and moreover they render these compounds resistant to enzymes.

In 1895, Fischer observed that sugars combine with one or two molecules of acetone and also with benzaldehyde to form well-defined isopropylidene and benzylidene compounds containing the groups:





Since 1901, Purdie and Irvine have employed methylation, either by methyl iodide and silver oxide or, more generally, dimethyl sulphate and caustic soda, to introduce stable methyl groups into all the hydroxyl positions of reducing sugars or into any hydroxyl groups which remain unsubstituted in a sugar derivative. The sequence of operations leading to a fully methylated glucose may be expressed by the following scheme:



The first stage is the formation of methylglucoside, a reaction which protects the reducing group, and this is followed by the introduction of methyl groups into the remaining hydroxyl positions. Acid hydrolysis eliminates the glucosidic alkyl group only, with the result that a tetramethylglucose is produced. Extending these principles, it is clear that if a sugar is substituted by any group or groups capable of subsequent removal by hydrolysis, it is possible to methylate the unoccupied hydroxyl positions and ultimately to obtain definite partly methylated sugars, and this method has been extensively employed in the study of the di- and poly-saccharoses.

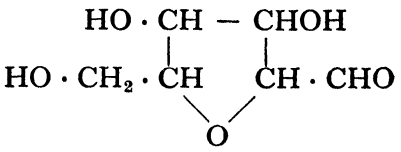
In general, it may be said that alkylated sugars are very suitable for exact and critical experimental study. In many cases the compounds crystallize readily in highly characteristic forms, and, in addition, the sugars or their glucosides can be effectively purified by distillation in a high vacuum. Moreover, the presence of the alkyl groups increase the solubility in organic solvents.

The Glucosamines.—In view of the manifold parts played by the substituted amino group in animal and vegetable metabolism, it is remarkable that few amino-derivatives of the sugars are known. In 1878, Lederhouse isolated an amino-sugar from lobster shells. This compound has the simple empirical relationship to glucose expressed by interchange of one hydroxl group in the glucose molecule for one amino group.

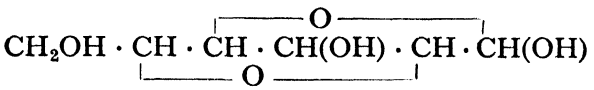
Both the hydrochloride and the pentacetate of glucosamine exist in two forms. It is reasonable to conclude that if glucosamine is treated with nitrous acid, glucose will be obtained, but in reality dehydration occurs and a sugar named chitose is obtained.

Fischer and Andrae * claim that chitose is a hydrated furfuran derivative rather than a true sugar:

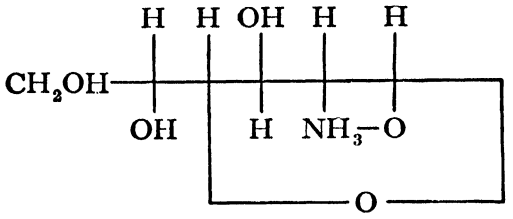
* *Ber.*, 1903, 36, 2587.



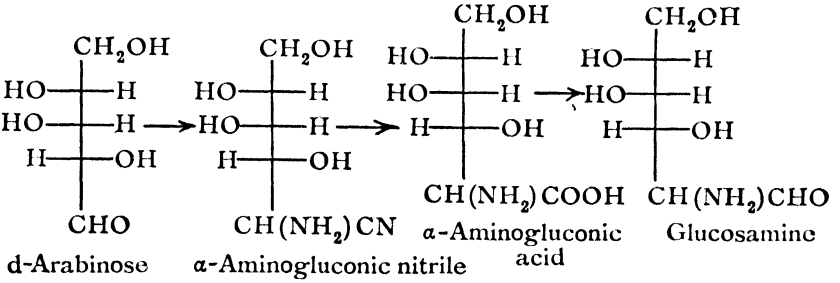
while Irvine and Hynd formulate chitose, with the aldehydic radical present, as in the hexoses, in the butylene-oxide form:



These authors * have succeeded in converting glucosamine into glucose by a long series of operations, and represent glucosamine by a formula of the betaïne type:



Fischer and Leuchs † have synthesized glucosamine from *d*-arabinose by the following reactions:



A second glucosamine was obtained by Fischer and Zach ‡ by the action of liquid ammonia on triacetylmethylglucoside, but its constitution is not known with certainty.

This branch of sugar chemistry retains a somewhat perplexing aspect, and this is all the more regrettable in view of the great biochemical interest attached to glucosamine as a connecting link between carbohydrates and amino acids.

Glucal.—In 1913, Fischer§ obtained a strongly reducing compound, $C_6H_{10}O_4$, which he named glucal, by the reduction of

* *Trans.*, 1912, **101**, 1128. † *Ber.*, 1903, **36**, 84.
‡ *Ber.*, 1911, **44**, 132. § *Sitz. Preuss. Akad. Wiss. Berlin*, 1913, 311.

β-acetobromoglucose with zinc dust and acetic acid. It is a slightly sweet, soluble syrup with aldehydic properties, and evidently possesses ethylenic unsaturation, since it decolorizes bromine water. The constitution of glucal has not been conclusively proved, but its properties are satisfactorily explained by the formula: *

$$HO \cdot CH_2 \cdot CH(OH) \cdot \underbrace{CH \cdot CH(OH) \cdot CH:CH}_{O}$$

The Natural and Artificial Glucosides.—The term glucoside is applied to a large number of substances having the property in common of furnishing a sugar (usually glucose) and one or more other products on hydrolysis. Glucosides occur in all parts of plants, but especially in the fruit, bark, and roots. The extraction is usually effected either by water or alcohol. In the former case it is first necessary to destroy the enzyme which accompanies the glucoside, or the latter may be hydrolyzed during the extraction.

Glucosides are generally colourless crystalline solids, having a bitter taste and lævorotary optical power. In chemical structure they resemble the simple α- and β-methylglucosides, and may therefore be represented by the general formula:

$$CH_2OH \cdot CHOH \cdot \underbrace{CH \cdot [CHOH]_2 \cdot CH}_{O}\text{–O–R}$$

where R is an organic radical.

The glucosides are all hydrolyzed by heating with mineral acids, and in the majority of cases they may also be hydrolyzed by suitable enzymes. The appropriate enzyme is contained in the same plant tissue but in different cells, gaining access to the glucoside only when the tissue is destroyed. The best known glucoside-splitting enzymes are the emulsin of almonds and the myrosin of black mustard seed. Emulsin is also able to act upon certain synthetic glucosides. It has already been mentioned that by the action of various alcohols upon sugars in the presence of hydrochloric acid, Emil Fischer was able to prepare two series of stereoisomeric glucosides, and that the α-glucosides are exclusively attacked by maltase whereas the β-glucosides are exclusively attacked by emulsin.

From these results it has been possible to draw conclusions as to the configurations of some of the natural sugars and glucosides. Maltose is an α-glucoside, for it is hydrolyzed by maltase and not by emulsin. Emulsin brings about the hydrolysis of lactose, from which it is evident that this sugar is related to the β-glucosides. Alkyl

* *Ber.*, 1920, **53** [B], 509.

glucosides derived from non-fermentable sugars are unattacked by both maltase and emulsin.

The better known glucoside-splitting enzymes are shown in the following table.

ENZYMES CAPABLE OF HYDROLYZING GLUCOSIDES

Enzyme.	Hydrolyses.	Products.
Emulsin.. ..	Many natural and synthetic glucosides.	
Prunase	Prunasin	Glucose, *d*-mandelonitrile.
Amygdalase ..	Amygdalin	Glucose, *d*-mandelonitrile glucoside.
Gaultherase ..	Gaultherin	Methyl salicylate, glucose.
Tannase ..	Tannins	Gallic and ellagic acids, and glucose.
Rhamnase ..	Xanthorhamnin ..	Rhamnitin, rhamninose.
Myrosin ..	Sinigrin	Allylthiocyanate, Potassium hydrogen sulphate.
Indigo ferment	Indican	Indoxyl and glucose.

The majority of the glucosides are derived from dextroglucose, but in addition glucosides derived from *d*- and *l*-arabinose, *d*-xylose, and *d*-ribose, from rhamnose and other methyl pentoses, and from galactose, mannose, and fructose, are known. In the glucosides all types of organic substances are united to glucose; for example, alcohols, aldehydes, acids, phenols, &c. A number of the better known glucosides are given in the table on p. 66, and frequent reference will be made to some of these compounds throughout this book.

The Structure of the Glucosides. — Three main points should be taken into account in the study of a glucoside. In the first place, the constituent sugar and the group with which it is combined must be identified, and the actual union of these compounds must be determined. This may be deduced from an examination of the products of hydrolysis, either by dilute acids or the action of an enzyme, but when the non-sugar residue contains several hydroxyl groups the structure arrived at by such means is open to doubt. In the second place, the particular configuration of the glucoside must be determined, for the compound may exist in the α- or β-stereoisomeric forms. This point may generally be settled by the study of enzyme action, for, since emulsin is the specific enzyme for β-glucosides, it may reasonably be concluded that all glucosides hydrolyzed by it are derived from β-glucose.

Glucoside.	Products of Hydrolysis.
	Phenols.
Arbutin	Glucose + hydroquinone.
Phloridzin	Glucose + phloretin.
Baptisin	Rhamnose + baptigenin.
	Alcohols.
Coniferin	Glucose + coniferyl alcohol.
Populin	Glucose + saligenin + benzoic acid.
Salicin	Glucose + saligenin.
	Aldehydes.
Amygdalin ..	2 mols Glucose + *d*-mandelonitrile.
Helicin	Glucose + salicylaldehyde.
Linamarin ..	Glucose + acetonecyanhydrin.
Prulaurasin ..	Glucose + racemic mandelonitrile.
Sambunigrin ..	Glucose + *l*-mandelonitrile.
	Acids.
Gaultherin ..	Glucose + methyl salicylate.
	Oxyflavone Derivatives.
Apiin	Apiose + apigenin.
Isoquercitin ..	Glucose + quercetin.
Xanthorhamnin ..	2 mols Rhamnose + galactose + rhamnetin.
	Mustard Oils.
Sinigrin	Glucose + allyl isothiocyanate + $KHSO_4$.
	Anthocyanins.
Cyanin	2 mols Glucose + cyanidin.
Delphinin	2 mols Glucose + *p*-hydroxy benzoic acid + delphinidin.
Idaein	Galactose + cyanidin.

The last point to be settled is the nature of the sugar residue. We have seen that the simple sugars and their derivatives may exist in modifications other than the ordinary butylene oxide type. Reliable evidence on this point cannot be obtained from a study of the sugar resulting from the hydrolysis of the glucoside, for rearrangement of the free hydroxyl groups of the carbohydrate may occur during this process. Trustworthy information regarding the internal structure of the sugar constituents may most readily be obtained by a study of the products of hydrolysis of the alkyl derivatives of the glucosides, and the actual hydroxyl group involved in glucoside formation may also be determined by such methods, when the non-sugar residue is a substance containing several such groups.

Irvine and Rose * obtained a pentamethyl salicin by methylation

* *Trans.*, 1906, 89, 814.

of the natural glucoside, and showed that the sugar in the parent glucoside possessed a butylene-oxide linking. They supported these observations by a synthesis of a pentamethyl salicin which proved to be identical with that derived from the natural glucoside by methylation.

More recently Macbeth and Pryde * have determined the structure of the glucoside indican, and this may be briefly considered. Earlier workers had shown that indican was an indoxyl glucoside, and that the constituent sugar was *d*-glucose, but no evidence regarding the internal linking of the sugar had been adduced. Indican was methylated, by the action of methyl iodide and dry silver oxide, and the resulting tetramethylindican hydrolyzed to tetramethylmethylglucoside and indoxyl derivatives. On hydrolysis of the former a crystalline tetramethylglucose was isolated which was readily identified as the 2 : 3 : 5 : 6 or butylene oxide compound. These results may be conveniently summarized:

Indican
↓
Tetramethyl indican
↙ ↘
Tetramethyl methylglucoside Indoxyl derivatives
↓
Tetramethyl glucose
(2:3:5:6)

From the results obtained it is evident that indican is derived from a molecule of *d*-glucose combined with indoxyl, the internal linking of the sugar being of the butylene-oxide type. The following structure is therefore established for the glucoside:

$$CH_2OH \cdot CHOH \cdot \underset{|\_\_\_\_\_\_\_\_\_\_}{CH} \cdot CHOH \cdot \underset{O}{} CHOH \cdot \underset{\_\_\_\_\_\_\_\_\_\_|}{CH} \cdot O \cdot C_8H_6N$$

and enzyme action and optical properties indicate that the compound is a derivative of *β*-glucose.

The Synthetic Glucosides.—Several of the natural glucosides have been prepared synthetically, and in addition a considerable number of artificial glucosides have been obtained, notably by Emil Fischer and his collaborators.

In 1879 Michael condensed crude acetochloroglucose with the potassium salts of various phenols, and in this way prepared phenyl

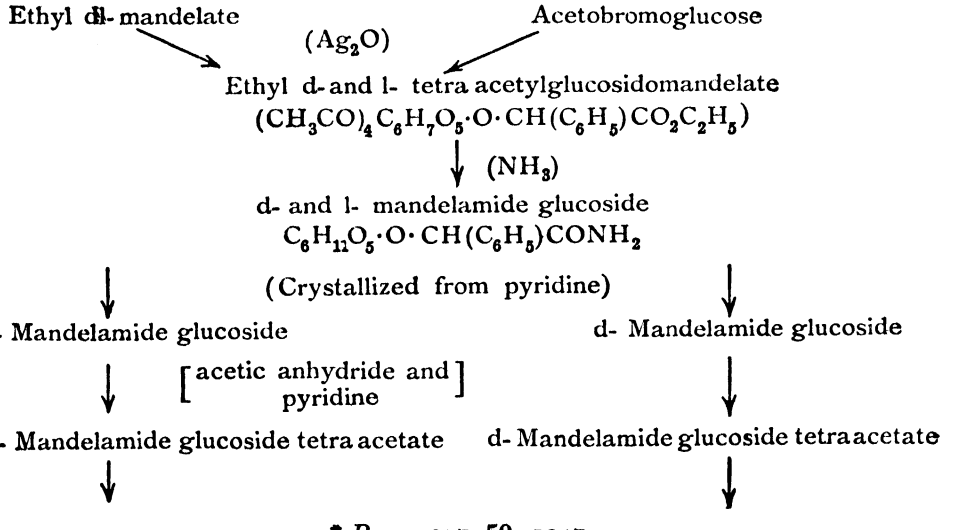
* *Trans.*, 1922, **122**, 1660.

glucoside, helicin, salicin, and methylarbutin. A more satisfactory method is to condense the non-saccharose constituent and acetobromoglucose in the presence of silver oxide. By an extension of this method purine glucosides (p. 208), terpene glucosides, and cyanophoric glucosides have been synthesized. A new modification of the glucoside synthesis consists in warming acetobromoglucose with phenol in the presence of quinoline. During this process a rearrangement takes place, and a mixture of α- and β-phenol glucosides is formed, which may be separated by crystallization from carbon tetrachloride. This synthesis of α-glucosides is of considerable importance, as hitherto it has been impossible to obtain them owing to the fact that α-acetochloroglucose gave rise to β compounds.

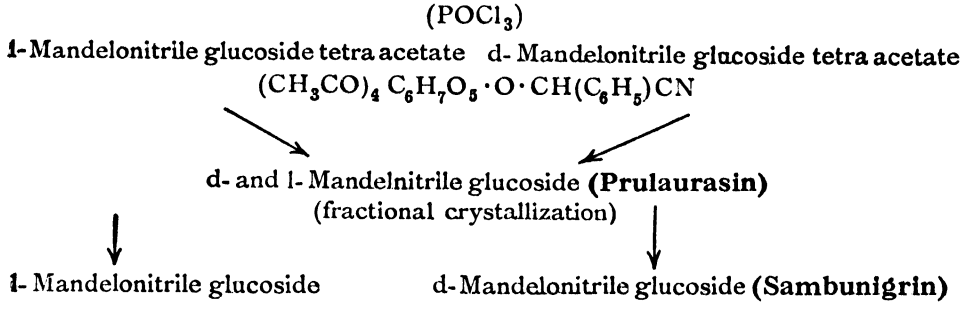
Considerable interest attaches to the synthesis of the glucosides containing hydrogen cyanide. This acid has been frequently isolated from plant products, but it is only quite recently that its formation has been ascribed invariably to the decomposition of a glucoside.

Amygdalin is perhaps the classic example of a glucoside, since it played such a conspicuous part in the early development of organic chemistry in the hands of Liebig and Wöhler. Even to-day its constitution is not established with certainty, but in all probability it is a derivative of a disaccharose.

Sambunigrin was obtained from the leaves of *Sambucus niger* by Bourquelot and Danjou in 1905, and in the following year Hérissey obtained prulaurasin from *Prunus laurocerasus*. Both these glucosides have been obtained synthetically by Fischer and Bergmann * according to the following scheme:



* *Ber.*, 1917, 50, 1047.



Soon after this Fischer described the synthesis of glycollonitrile-glucoside, the simplest of the cyanophoric glusocides, and that of linamarin (from flax), the glucoside of acetonecyanhydrin.

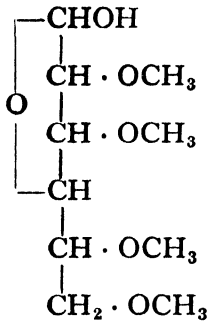
Finally it may be mentioned that Bourquelot has obtained glucosides synthetically by means of enzymes. Whereas in dilute aqueous solution the hydrolysis of β-methyl glucoside by emulsin is complete, hydrolysis is retarded by increasing amounts of methyl alcohol until in the presence of a certain proportion of this alcohol the enzyme is able to synthesize glucoside from glucose and the alcohol. The reaction has been extended to other alcohols, the enzyme being allowed to act on sugars dissolved in alcohols containing varying amounts of water or acetone. In this way crystalline glycol-, glycerol-, geranyl-, and cinnamyl-β-glucosides have been obtained by means of emulsin.

THE DISACCHAROSES

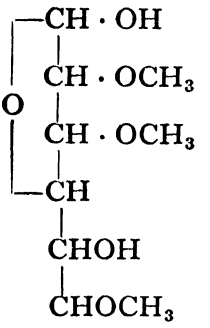
The disaccharoses are carbohydrates containing twelve carbon atoms, and consist of two simple six-carbon atom residues united through an oxygen atom. When hydrolyzed by acids or enzymes, one of the constituent hexoses functions in the same manner as glucose does in the methyl glucosides, while the aldehydic or ketonic group of the second hexose may remain functional or disappear. In the former case the disaccharose reduces cupric salts, exhibits mutarotation, and forms an osazone, e.g. maltose, lactose, and melibiose, while in the latter case the sugar has no reducing properties, e.g. sucrose and trehalose.

Research work on the disaccharoses therefore centres round three points: determination of the nature of the component hexoses, the type of glucosides which they represent, and the hydroxyl group concerned in the attachment.

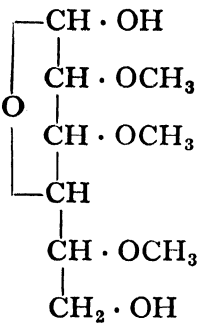
Irvine and his collaborators have employed five methylated hexoses as reference compounds to determine the constitution of the most important disaccharoses and polysaccharoses:



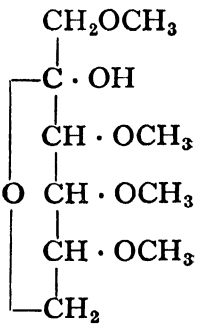
2:3:5:6-Tetramethyl glucose or galactose



2:3:6-Trimethylglucose



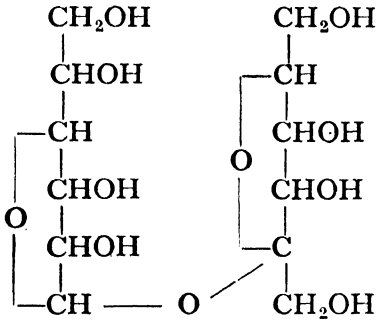
2:3:5-Trimethyl-glucose



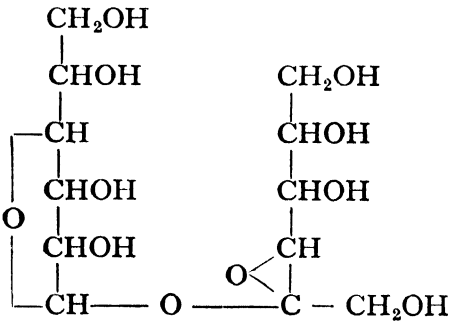
Tetramethyl γ-fructose

Of these, 2:3:5:6-tetramethylglucose has proved of greatest service.

Structure of Sucrose (Cane Sugar).—In conformity with the absence of reducing properties of cane sugar, Fischer put forward a formula (i) in 1893:



(i)



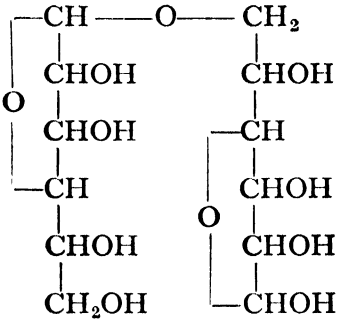
(ii)

This formula remained unchallenged until his isolation of γ-methylglucoside (p. 58), when he drew attention to the similar behaviour of these two substances towards acids. While it is assured that the glucose residue has the same type of oxide ring as that of the α- and β-glucosides, his inference was that the fructose component is in the γ form. This view has received confirmation by Haworth and Law,* who prepared octamethylsucrose and observed that when treated with dilute acid it gives tetramethylglucose and tetramethylfructose. The methylated aldose proved to be the tetramethylglucose of the butylene oxide type, while the ketose

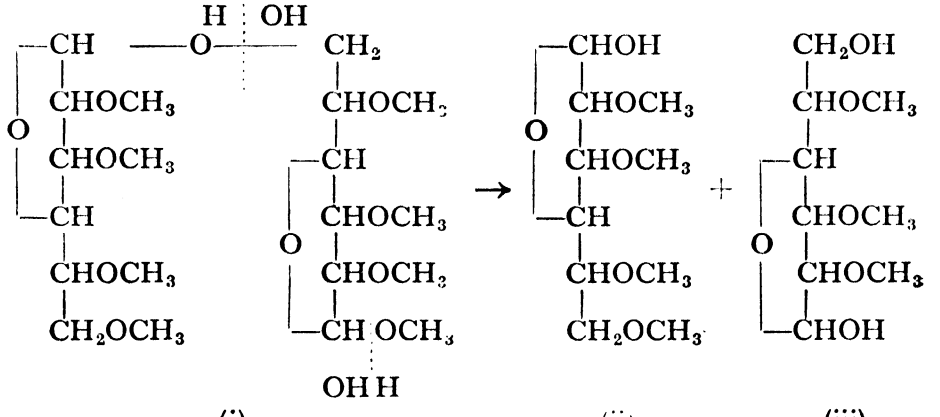
* *Trans.*, 1916, **109**, 1314.

displayed a rotatory power and reaction towards permanganate which at once stamped it as being allied in structure to " γ-glucose ". The new provisional formula (ii) shows a butylene oxide aldose coupled to an ethylene oxide ketose through their reducing groups.

Maltose.—Maltose is a reducing sugar which yields, on hydrolysis with dilute acids or with maltase, two molecular proportions of glucose. Consequently it is regarded as a biose having the reducing group of one glucose molecule united through an anhydride linking with a second glucose residue. The constitution assigned to maltose by Fischer was:



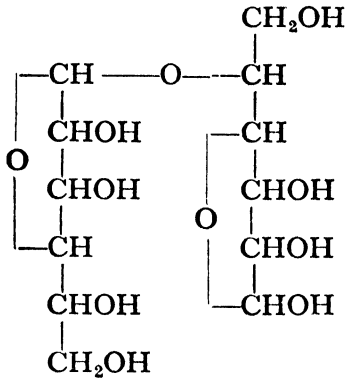
This structure has been confirmed by Haworth and Miss Leitch.* Starting from the free sugar, methyl maltoside was produced by the regulated action of methyl sulphate and sodium hydroxide, and the same reagents were then used to convert this product into heptamethyl-methylmaltoside (i). On hydrolysis, the tetramethylglucose (ii) and the trimethylglucose (iii) were obtained.



(i) (ii) (iii)

* *Trans.*, 1919, **115**, 809.

Cellobiose.—In the same paper the following formula was suggested for cellobiose:



Haworth and Hirst * have prepared this sugar from cellulose (p. 79) and converted it into an octamethyl derivative which was shown to be heptamethyl-methylcellobiose. The hydrolytic products obtained from this compound are in accordance with the above formula for cellobiose.

Lactose.—Lactose or milk sugar is present in the milk of all mammalia, but it has not been found in the vegetable kingdom. The preparation of lactose from milk is easily carried out. For this purpose rennet is added to milk to coagulate the casein, and the clear liquid or " whey " which separates is concentrated in vacuo.

It is interesting to note that lactose was the first sugar of which the occurrence of more than one modification was observed. Three forms are known, and are designated α, β, and γ, the last being an equilibrium mixture of the α and β forms. Hudson † has made a careful study of the modifications of lactose, and his papers should be consulted for further details.

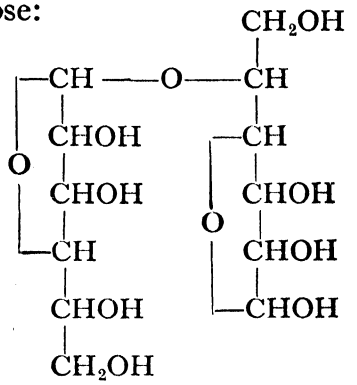
Lactose resembles cane sugar and maltose in forming esters with eight equivalents of acid. Haworth and Miss Leitch ‡ have investigated the constitution of lactose in a similar manner to that already described in the case of sucrose. Lactose was completely methylated and then hydrolyzed, when products were obtained according to the following scheme:

Lactose → methyl lactoside → heptamethyl-methyl lactoside.

→ Tetramethylhexose (A).
→ Methylalcohol.
→ Trimethylhexose (B).

* *Trans.*, 1921, **119**, 193. † *J. Amer. Chem. Soc.*, 1908, **30**, 1767.
‡ *Trans.*, 1918, **113**, 188.

Compound (A) was shown to be the butylene oxide form of tetramethyl galactose, while (B) was shown to be identical with the trimethyl glucose isolated by Denham from methylated cellulose (p. 79). From this evidence the following structural formula has been assigned to lactose:



[Galactose residue] [Glucose residue]

Lactose

Trehalose, mycose, or mushroom sugar was discovered by Wiggers, in 1832, in ergot, and has subsequently been found to be a constituent of most mushrooms, toadstools, and other fungi. It appears to replace sucrose in those plants which do not contain chlorophyll and do not elaborate starch. It is also found formed in trehala manna, a cocoon formed by certain species of beetles on several spiny plants native to Syria and Persia.

The hydrolysis of trehalose with dilute mineral acids takes place very slowly and produces a quantitative yield of *d*-glucose. Maltase, invertase, emulsin, and diastase are without action on trehalose, but it is readily hydrolyzed by the enzyme trehalase, which is conveniently obtained from the fungus *Aspergillus niger.*

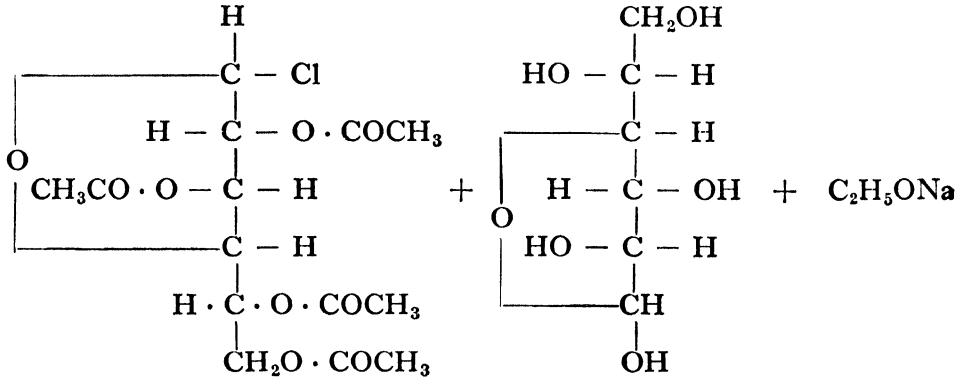
Trehalose does not reduce Fehling's solution and does not form either hydrazones or osazones.

Melibiose has not been found naturally in the free state, but is produced by the hydrolysis of raffinose:

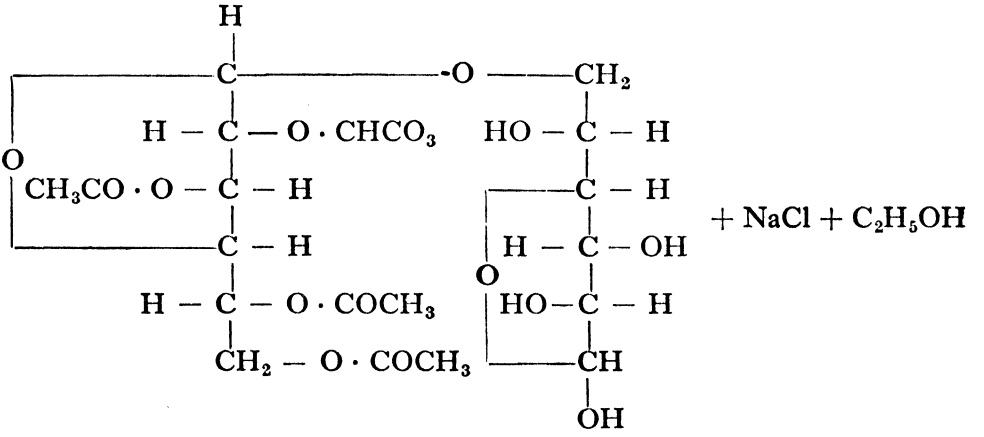
$$\underset{\text{Raffinose}}{C_{18}H_{32}O_{16}} + H_2O = \underset{d\text{-Fructose}}{C_6H_{12}O_6} + \underset{\text{Melibiose}}{C_{11}H_{22}O_{11}}$$

Melibiose is reduced by sodium amalgam to melibitol, which on hydrolysis gives mannitol and *d*-galactose. It is hydrolyzed by strong acids to *d*-glucose and *d*-galactose. With emulsin the hydrolysis is slow, but melibiase, an enzyme found in bottom yeast, attacks this disaccharose rapidly. It reduces Fehling's solution and forms hydrazones and osazones.

Melibiose was the first disaccharose to be obtained synthetically. It was prepared by Fischer and Armstrong * from acetochlorogalactose and sodium glucosate, which condense to give melibiose tetracetate. On hydrolysis with caustic soda, melibiose is obtained.



Acetochlorogalactose Glucose



Melibiose tetracetate

Turanose and **Gentibiose** are obtained by the hydrolysis of the trisaccharoses, melecitose and gentianose, respectively.

THE TRISACCHAROSES

Raffinose, $C_{18}H_{32}O_{16}$, is the best known and most widely distributed trisaccharose. It was first isolated by Johnston in 1843 from eucalyptus manna, and was later obtained from beet sugar in the refining process by Loiseau, who gave to it the name raffinose (*raffiner* = to refine). It is most conveniently prepared from cotton-seed meal by precipitation from an aqueous extract with calcium

* *Ber.*, 1902, 35, 3144.

oxide and subsequent decomposition of the calcium salt with carbon dioxide.

Raffinose exhibits no reducing properties. On hydrolysis with dilute acids it gives *d*-fructose, *d*-glucose, and *d*-galactose:

$$\underset{\text{Raffinose}}{C_{18}H_{32}O_{16}} + 2H_2O = \underset{d\text{-Fructose}}{C_6H_{12}O_6} + \underset{d\text{-Glucose}}{C_6H_{12}O_6} + \underset{d\text{-Galactose}}{C_6H_{12}O_6}$$

In practice the hydrolysis takes place in two stages, melibiose (p. 73) and fructose being the first products, and the melibiose then yielding galactose and glucose. Invertase hydrolyzes raffinose to fructose and melibiose, while emulsin breaks it down to sucrose and galactose. From these observations the following formula may be constructed:

$$\underbrace{\underbrace{C_6H_{11}O_5}_{\text{Fructose}} - O - \underbrace{C_6H_{10}O_4}_{\text{Glucose}}}_{\text{Sucrose}} - O - \underbrace{\underbrace{C_6H_{11}O_5}_{\text{Galactose}}}_{\text{Melibiose}}$$

Gentianose was discovered by Meyer in 1882 in Gentian roots (*Gentiana lutea*), and is most conveniently prepared by extraction of the dried roots with 95 per cent alcohol. Gentianose is a non-reducing sugar which possesses a slightly sweet taste. On hydrolysis it splits up into fructose and two molecules of glucose, or, in stages, with the formation of either fructose and gentiobiose (by gentianase) or of sucrose and glucose (by emulsin).

$$\underbrace{\underbrace{C_6H_{11}O_5}_{\text{Fructose}} - O - \underbrace{C_6H_{10}O_4}_{\text{Glucose}}}_{\text{Sucrose}} - O - \underbrace{\underbrace{C_6H_{11}O_5}_{\text{Glucose}}}_{\text{Gentiobiose}}$$

Melecitose, or melezitose, was first observed by Bonastre in 1833 in the manna from the larch (*Pinus larix*). It is prepared from Turkestan manna by extraction with warm water and crystallization from methyl alcohol. It does not form osazones and is not a reducing sugar.

On hydrolysis with dilute acids it gives glucose and turanose, the latter being further hydrolyzed to glucose and fructose. The manner of arrangement of the two glucose and one fructose residues in this sugar is unknown.

Mannotriose was found by Tanret in 1902 in the manna of the ash (*Fraxinus ornus*). The manna contains up to 16 per cent of mannotriose, up to 60 per cent of mannitol, and small quantities of stachyose, and the separation of these sugars is tedious.

Mannotriose reduces Fehling's solution and forms a phenyl-

osazone. On hydrolysis with dilute acids the sugar yields two molecules of galactose and one of glucose, while emulsin gives glucose and digalactose:

$$\underset{\text{Glucose}}{OHC \cdot C_5H_{10}O_4} - O - \underbrace{\underset{\text{Galactose}}{C_6H_{10}O_4}}_{\text{Gluco-galactose}} - O - \underbrace{\underset{\text{Galactose}}{C_6H_{11}O_5}}_{\text{Digalactose}}$$

Rhamninose, $C_{18}H_{32}O_{14}$, along with rhamnetin, is a product of the hydrolysis of the glucoside xanthorhamnetin, found in the Persian berry (*Rhamnus infectoria*). The hydrolysis is effected by the enzyme rhamninase, which is present in the berries.

Rhamninose is a reducing sugar which possesses a slightly sweet taste. On hydrolysis with dilute acids it gives one molecule of *d*-galactose and two molecules of rhamnose:

$$\underset{\text{Galactose}}{OHC \cdot C_5H_{10}O_4} - O - \underset{\text{Rhamnose}}{C_6H_{10}O_3} - O - \underset{\text{Rhamnose}}{C_6H_{11}O_4}$$

TETRASACCHAROSES

Stachyose, lupeose, or mannotetrose, $C_{24}H_{42}O_{21}$, was discovered by Planta in 1888 in the tubers of *Stachys tubifera*, in which it is sometimes present to the extent of 70 per cent. Its occurrence in the manna of the ash has already been mentioned, and it has also been found in the roots of various Labiatæ.

It is not a reducing sugar. On hydrolysis with weak acids or invertase it yields *d*-fructose and mannotriose:

$$\underset{\text{Stachyose}}{C_{24}H_{42}O_{21}} + H_2O = \underset{d\text{-Fructose}}{C_6H_{12}O_6} + \underset{\text{Mannotriose}}{C_{18}H_{32}O_{16}}$$

Stronger acids hydrolyze stachyose to the hexoses. According to Biérry * the gastro-intestinal juice of *Helix pomatia* effects the hydrolysis in the following stages:

(I) To *d*-fructose and mannotriose.
(II) Mannotriose to *d*-galactose and a disaccharose.
(III) The disaccharose into galactose and glucose.

The formula of stacyhose may thus be provisionally represented:

$$\underset{\text{Fructose}}{C_6H_{11}O_5} - O - \underbrace{\underset{\text{Glucose}}{C_6H_{10}O_4} - O - \underset{\text{Galactose}}{C_6H_{10}O_4} - O - \underset{\text{Galactose}}{C_6H_{11}O_5}}_{\text{Mannotriose}}$$

* *Biochem. Zeitsch.*, 1912, **44**, 446.

THE POLYSACCHAROSES

The polysaccharoses are substances of high molecular weight, and most of them are amorphous and insoluble in water. On hydrolysis they break down into sugars containing five or six carbon atoms, and may therefore be regarded as anhydrides of these substances. Since we have no reliable knowledge of the molecular weights of these compounds, their formulæ are written $(C_6H_{10}O_5)_n$ or $(C_5H_8O_4)_n$, according as they give rise to hexoses or pentoses on hydrolysis.

The polysaccharoses may be classified as follows:

1. Starches and dextrins, including glycogen, inulin, &c.
2. Gums, which comprise (*a*) natural gums and pentosans, and (*b*) mucilages and pectic bodies.
3. Celluloses.

The importance of these substances cannot be overestimated and yet we have very little knowledge of the chemical constitution of any of them. Much might be written on the importance of these substances from the chemical, botanical, physiological, and commercial points of view, and indeed, in spite of our limited knowledge, the most remarkable devices have been successfully employed for the utilization of these substances, and especially the derivatives of cellulose, in industry. The consideration of these numerous applications is beyond the scope of this book, and the reader desiring information on these matters should consult one of the numerous treatises, of which Worden's *Technology of Cellulose Esters* is perhaps the most monumental.

During the last few years the chemistry of cotton cellulose has received considerable attention, and since more progress has been made in the study of this polysaccharose than the other members of this group, it will be well to consider the celluloses first of all.

The Celluloses.—The term cellulose should be taken in general to connote a group of substances rather than a single chemical compound, and used in this generic sense we may classify the celluloses as follows.

1. Normal or typical celluloses of the cotton type, e.g. the cellulose obtained from cotton, flax, hemp, &c.

2. Compound celluloses of the wood cellulose, jute, and cereal grass types. The natural celluloses occurring in jute, cereal straws, esparto, &c., consist of some form of cellulose combined with a non-cellulose constituent which may be either of the nature of lignin (e.g. jute fibre), a pectic or gummy substance (e.g. flax), or a fatty substance (e.g. cork).

3. Hemi-, pseudo-, or reserve celluloses, which represent a very heterogeneous collection of substances, are much more easily hydrolyzed than other celluloses and give rise to various sugars such as mannose, galactose, and certain pentoses. These celluloses occur in the cell walls of the seeds of various plants, e.g. *Soja hispida*, *Cocos nucifera*, beans, peas, &c.

Cotton Cellulose.—The extensive study of the alkylated sugars (p. 61), with which the names of Purdie, Irvine, and Haworth are intimately connected, has opened out a hopeful method for the determination of the constitution of the polysaccharoses. Now that the properties and structure of a large number of alkylated aldoses and ketoses are known, the substances formed in the degradation of the alkylated polysaccharoses may be identified.

The original method, employing silver oxide and methyl iodide, is not always successful owing to the experimental difficulties frequently caused by the insolubility of the carbohydrate in methyl iodide, or to the presence of reducing sugars when the silver oxide functions as an oxidizing agent, and in these cases, methylation by means of dimethyl sulphate and caustic soda gives better results. Investigations of this type must include, (1) the identification of the constituent sugars, (2) their stereochemical form, (3) the hydroxyl group involved in the coupling of the constituents, and (4) the position of the internal oxygen atom in each ring.

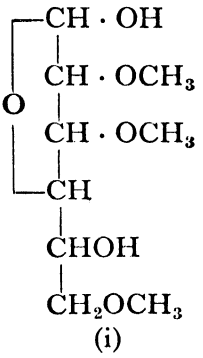
These methods give more certain results than the investigation of the acyl derivatives * of the sugars, or the acetolysis † of the polysaccharoses by the action of acetyl bromide in the presence of hydrobromic and acetic acids, since the latter brings about simultaneous acetylation, hydrolysis, and bromination.

Willstätter ‡ regards cellulose as a polyglucose, and has claimed that the complex may be quantitatively transformed into glucose. This work has, however, been repeated by Miss Cunningham,§ who has shown that it is impossible to estimate, by means of the polari-

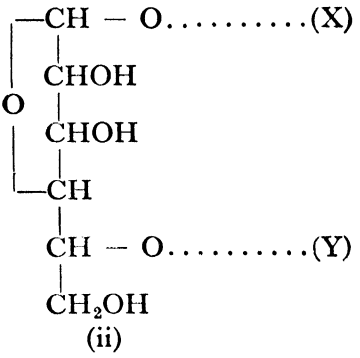
* Hess and Messmer, *Ber.*, 1921, **54** B, 499.
† Bergmann and Beck, *ibid.*, 1574.
‡ *Ber.*, 1913, **46**, 2401. § *Trans.*, 1918, **113**, 173.

meter, the amount of glucose produced in a system saturated with hydrochloric acid, as this acid produces profound constitutional changes in the sugar. Estimations based on reducing power are equally valueless.

Denham and Woodhouse* succeeded in alkylating cotton cellulose, and obtained a derivative which on hydrolysis yielded a mixture of methylated products from which 2 : 3 : 6-trimethylglucose (i) was isolated.



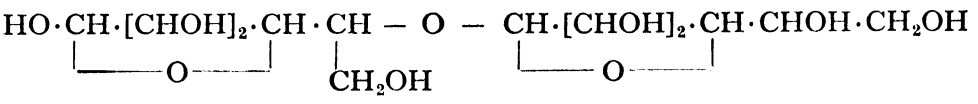
(i)



(ii)

This trimethylglucose has been obtained from several other methylated sugars and its structure is well established.† This work gave the first clear evidence as to the linkage of part of the cellulose molecule which must contain the unit shown in formula (ii).

Irvine and Soutar‡ continued this work, and by the degradation of a purified methylated cellulose obtained an 85 per cent yield of crystalline derivatives of glucose. The cellulose was treated with acetic anhydride and sulphuric acid, and both the soluble and insoluble portions were converted into methylglucoside, quite free from any isomeric methylhexoside. These results clearly showed the absence of mannose and galactose residues in cellulose, and pointed to the following formula for cellobiose:



Haworth and Hirst § obtained cellobiose in a yield of 30 per cent by an improved method of acetolysis of cellulose, and, as already shown (p. 72), confirmed the above structural formula.

* *Trans.*, 1914, **105**, 2357.
† *Trans.*, 1922, **121**, 1213.
‡ *Trans.*, 1920, **117**, 1489.
§ *Trans.*, 1921, **119**, 293.

More recently Irvine and Hirst * have shown that the cellulose molecule is entirely composed of glucose residues by obtaining an over-all yield of glucose derivative equal to 95 per cent of that theoretically available.

Cotton cellulose, 100 parts .. ..
↓
Cellulose triacetate, 177 parts .. .. } 99·5 per cent
(Methyl alcohol containing 0·75 per cent HCl)
↓
α- and β-methylglucosides, 114·1 parts } 95·5 per cent
Glucose equivalent, 106 parts

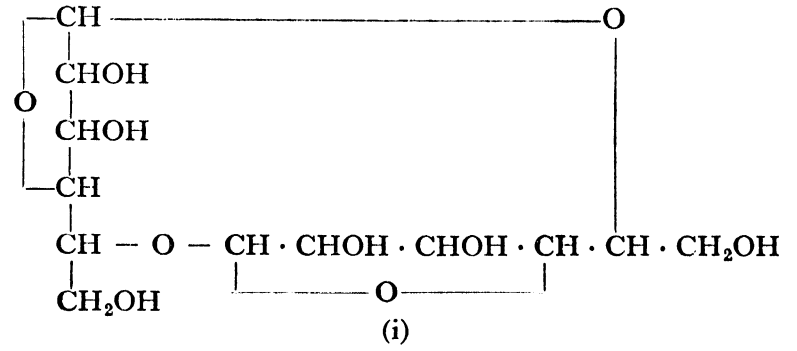
Denham and Woodhouse's work was continued by Irvine, Denham, and Hirst, and by the exhaustive methylation of cotton cellulose a product was obtained which contained 43·0 per cent of methoxyl in place of 45·6 per cent required by the trimethyl derivative. The material still preserved its fibrous nature, from which it was concluded that no profound molecular change had taken place. The trimethylcellulose was then submitted to simultaneous depolymerization, hydrolysis, and conversion of the scission products into the corresponding methylglucosides. These were distilled in a high vacuum, and the distilled material consisted of 2 : 3 : 6-trimethyl methylglucoside only. On hydrolysis of the distilled glucoside crystalline 2 : 3 : 6-trimethylglucose alone was obtained. These reactions may be summarized:

Cotton cellulose
↓
Trimethyl cellulose .. .. .. } yield 90 per cent.
↓
2-, 3-, 6-Trimethyl methylglucoside
↓
2-, 3-, 6-Trimethyl glucose .. .. } yield 89 per cent

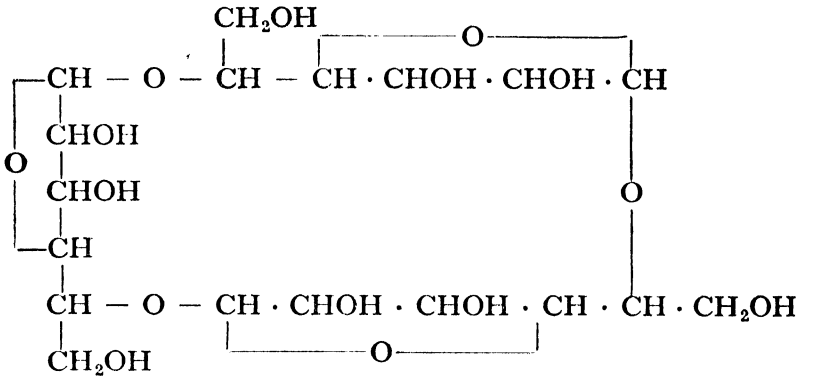
The scheme affords a proof that all the glucose residues in cellulose are identical in structure and have the hydroxyl groups 2, 3, and 6 unsubstituted. To satisfy this condition and to account for the formation of cellobiose, it is necessary to include in the formula at least two glucose residues (i). In view of the fact that the highest yield of cellobiose so far obtained does not approach that theoretically

* *Trans.*, 1922, **121**, 1585.

possible on the bases of formula (i), an alternate formula (ii), in which the symmetrical tri-1 : 5-anhydroglucose is the unit of cellulose, appears to be the more favourable.*



(i)



(ii)

Starches and Dextrins.—Starch is one of the most widely distributed substances in the vegetable kingdom. As a more or less permanent reserve food material it occurs in seeds, fruits, the vegetable parts such as tubers, &c., and in the latex of certain plants. It also occurs in green leaves, presumably as a temporary reserve material.

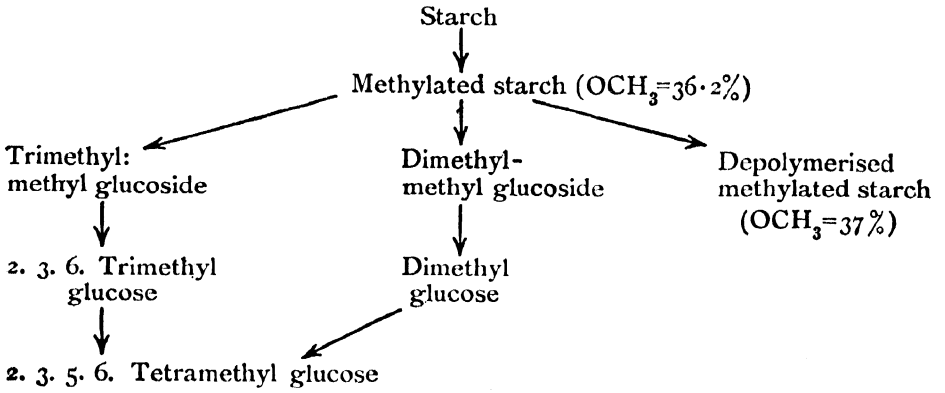
The preparations, properties, and uses of starch need not be dealt with here, and in spite of the amount of work which has been devoted to the study of its chemical constitution our knowledge of the nature of the starch molecule is very slight indeed.

Irvine and Macdonald † have shown that when starch is repeatedly methylated the reaction ceases when the methoxyl content is 37 per cent. This value corresponds exactly with the theoretical amount calculated on the basis that one hexose residue has acquired

* Irvine, *J. Soc. Chem. Ind.*, 1922, **41**, 362 R.

† *J. Soc. Chem. Ind.*, 1922, **41**, 362 R.

three methyl groups whilst four are shared by two glucose residues. When digested with methyl alcohol containing hydrochloric acid, the methylated starch was converted into trimethyl-methylglucoside and dimethyl-methylglucoside. These were separated by distillation in high vacuum and thereafter hydrolyzed to give the parent sugars. An unexpected result was encountered in that the trimethylglucose isolated proved to be the crystalline form in which the methyl groups occupy the 2:3:6 positions. One glucose residue in starch must thus be substituted as shown in formula (ii) (p. 79). In order to accommodate the formation of maltose from starch, either one or two additional glucose residues must be present at X and Y in the unit. The reactions involved may be summarized:



The work so far carried out has not been sufficient to justify a formula which will fit in with these facts and at the same time account for all the properties of the starch molecule.

Inulin is of common occurrence as a reserve food-stuff. It is very conveniently prepared from dahlia tubers, and in many of its properties resembles starch. It is derived from fructose, and until recently there was no reason to doubt that the parent hexose was the well known lævorotatory form of the ketose. Inulin has been submitted to examination on similar lines to that already described for cellulose and starch, by Irvine and his collaborators.

Very little is known of the chemical nature of the various natural gums and mucilages. The chemistry of the polysaccharoses offers an exceedingly wide field for future chemical research, and it is most desirable that the chemist should obtain a knowledge of the constitution of these important natural compounds.

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CHAPTER IV

The Depsides, Lichen Products, and Tannins

Introduction.—In very early times it was known that certain parts of plants possess a very astringent taste, give a black coloration with substances containing iron, and have the property of converting raw hides into leather. Some writers employ the term " tannin " as the generic name for this widely disseminated group of vegetable products, while others have unfortunately used it to denote a particular substance better described as gallotannin.

Gallic acid was prepared by Scheele in 1786 by exposing nut galls to the air, in a warm place, and frequently removing the film of mould. Scheele's product was undoubtedly very impure, and gallic acid was first obtained in an almost pure state by Berzelius. Even earlier than this, Lewis, in 1763, had isolated gallotannin, and five years later Piepenbring had obtained gallic acid from it.

Gallotannin, obtained from gall apples, has been the subject of many investigations. In 1852 Strecker denoted gallotannin by the formula $C_{27}H_{22}O_{17}$, and considered it as a compound of one molecule of grape sugar and three molecules of gallic acid. In 1871 Schiff denoted a tannin-like product—which Löwe had obtained by heating gallic acid with arsenious acid—as digallic acid, and for a long time afterwards gallotannin was assumed to be identical with digallic acid. This erroneous view was finally shattered by Flawitzki, who, in 1895, showed that gallotannin was optically active.

The subsequent development of the chemistry of gallotannin culminated in 1918 with the synthesis of the active gallotannin from Chinese tannin by Emil Fischer. In the course of his investigations, Fischer not only synthesized compounds with tannin-like properties, which he termed " Depsides " ($\delta\epsilon\psi\epsilon\iota\nu$ = to tan), but he also synthesized two lichen products—Lecanoric and Evernic acids.

Classification.—Owing to our present incomplete knowledge of the chemical constitution of the tannins, it is difficult to evolve a

proper chemical classification of these substances. Proctor * classifies the tannins in two main groups:

1. **Pyrogallol Tannins,** including divi-divi, galls, oak-wood, and chestnut tannins. These tannins give a dark blue colour with ferric salts, give no precipitate with bromine water, and on leather produce a " bloom " consisting of ellagic acid.

2. **Pyrocatechol Tannins,** including all the pine barks, acacias, mimosas, oak barks (but not oak wood, fruits, or galls), quebracho wood, cassia and mangrove barks, cutch, and gambia. These tannins give a greenish-black colour with iron alum, a yellow or brown precipitate with bromine water, and deposit no " bloom ". The addition of concentrated sulphuric acid to a drop of the infusion produces a dark red or crimson ring at the junction of the two liquids. Some of the tannins in this class contain phloroglucinol as one of the constituents of the molecule.

Isolation of the Tannins.—The majority of the tannins are soluble in hot water and may be precipitated with lead acetate. The plant infusion is treated with an aqueous solution of lead acetate and the precipitate decomposed, in the moist condition, with hydrogen sulphide. The solution of tannin thereby obtained is then concentrated in vacuo.

In many cases it is preferable to employ an organic solvent for extraction. Various mixtures of alcohol, water, and ether may be used, but acetone is probably the best solvent. Ethyl acetate is used extensively, but some tannin glucosides are insoluble in this solvent. The method used by Fischer and Freudenburg for the purification of Chinese tannin will be described later.

The Depsides.—The term " depside " was introduced by Fischer and Freudenburg † to denote a series of anhydrides formed by the condensation of a carboxylic group of a phenolcarboxylic acid with a hydroxyl group of the same or a similar acid, e.g.

$$\underset{\text{Hydroxybenzoic acid}}{HO\cdot C_6H_4COOH} + HOC_6H_4COOH = H_2O + \underbrace{HO\cdot C_6H_4CO\cdot O\cdot C_6H_4COOH}_{\text{(a didepside)}}$$

This product may condense with another molecule of a phenolcarboxylic acid to give a tridepside, $HO\cdot C_6H_4CO\cdot O\cdot C_6H_4CO\cdot O\cdot C_6H_4COOH$, and by an extension of this reaction tetradepsides may be obtained. The nomenclature is, indeed, very similar to that employed for the polysaccharoses and the polypeptides.

* *Principles of Leather Manufacture*, London, 1903. † *Ann.*, 1910, **372**, 35.

As early as 1883, Klepl had obtained di- and tri-depsides by heating *p*-hydroxybenzoic acid, and Schiff had prepared similar products by the action of dehydrating agents on phenolcarboxylic acids.

In order to obtain a satisfactory yield in the preparation of the depsides, it is necessary to protect the hydroxyl group of one of the acids undergoing reaction, and for this purpose Fischer first employed methylchloroformate * ($ClCOOCH_3$), e.g.

$$HO \cdot C_6H_4COOH + ClCOOCH_3 = CH_3CO_2 \cdot O \cdot C_6H_4COOH + HCl$$

In the case of those phenolic acids in which the hydroxyl group is in the meta or para position to the carboxyl group, this reaction may easily be brought about with the aid of caustic soda, but when the hydroxyl group is in the ortho position it is preferable to use dimethylaniline in an indifferent solvent for the removal of the elements of hydrochloric acid. This latter method was first devised by Fritz Hofmann in 1899. The following tabulation illustrates the applications of these methods of carbomethoxylation.

In aqueous solution.	*Dimethylaniline method.*
p-Hydroxybenzoic acid.†	Salicylic acid.§
m-Hydroxybenzoic acid.	α- and β-Hydroxynaphthoic acids.**
Vanillic acid.‡	β-Resorcylic acid.§
o-Cumaric acid.§	Phloroglucinol carboxylic acid.‖
Protocatechuic acid.†	
Orsellinic acid.**	
Gallic acid.†	

Partial carbomethoxylation may be accomplished in the case of polyhydroxyphenolic acids; e.g. using one molecule of chloroformic ester, the *p*-methylcarbonato or carbomethoxy derivatives of β-resorcylic acid and orsellinic acid were obtained, while in the case of gallic acid the meta derivative was first obtained.

The methylcarbonato group is easily removed by an excess of cold aqueous alkali, and more slowly, as urethane, by a normal solution of ammonia. In some cases a selective removal of a methylcarbonato group may be accomplished.

The substituted phenolic acids readily react with phosphorus pentachloride to give acid chlorides in the usual way.

* Fischer had previously employed methylchloroformate for the protection of the hydroxyl group in tyrosine, in the preparation of tyrosylglycine.

† *Ber.*, 1908, **41**, 2877. ‡ *Ann.*, 1910, **372**, 47. § *Ber.*, 1909, **42**, 226.
‖ *Ann.*, 1912, **391**, 366. ** *Ber.*, 1913, **46**, 2400.

Before describing the synthesis of the depsides, two simple applications of these methods may be illustrated.

p-Hydroxyhippuric Acid.*—This acid was prepared from p-hydroxybenzoic acid and glycine ester in the following stages:

$$\underset{p\text{-Hydroxybenzoic acid}}{HO\cdot C_6H_4COOH} \xrightarrow{ClCO_2CH_3} CH_3\cdot CO_2\cdot O\cdot C_6H_4COOH \xrightarrow{PCl_5} CH_3CO_2\cdot O\cdot C_6H_4COCl$$

This acid chloride was then condensed with glycine ester, and on hydrolysis with caustic soda, p-hydroxyhippuric acid was obtained:

$$CH_3CO_2\cdot O\cdot C_6H_4COCl + 2NH_2\cdot CH_2\cdot CO_2C_2H_5$$
$$= CH_3CO_2\cdot O\cdot C_6H_4CO\cdot NH\cdot CH_2CO_2C_2H_5 + HCl, NH_2CH_2COOC_2H_5$$
$$\rightarrow HO\cdot C_6H_4\cdot CO\cdot NH\cdot CH_2COOH$$

p-Hydroxyhippuric acid.

p-Hydroxybenzophenone.†—This was synthesized from p-hydroxybenzoic acid as follows:

$$HO\cdot C_6H_4COOH \xrightarrow{ClCO_2CH_3} CH_3CO_2OC_6H_4COOH \xrightarrow{PCl_5} CH_3CO_2\cdot O\cdot C_6H_4COCl$$

The acid chloride was then condensed with benzene by the usual Friedel Craft reaction and the product hydrolyzed.

$$CH_3CO_2\cdot O\cdot C_6H_4COCl + C_6H_6 \rightarrow CH_3CO_2O\cdot C_6H_4CO\cdot C_6H_5$$
$$\rightarrow HO\cdot C_6H_4\cdot CO\cdot C_6H_5$$

In a similar way 2: 3: 4-trihydroxybenzophenone (alizarin yellow) was obtained from pyrogallolcarboxylic acid.

Didepsides.—The simplest didepside is obtained by the condensation of two molecules of p-hydroxybenzoic acid ‡ in cold alkaline solution as follows:

$$CH_3CO_2\cdot O\cdot C_6H_4COCl + NaO\cdot C_6H_4CO_2Na$$
$$= CH_3CO_2\cdot O\cdot C_6H_4CO\cdot O\cdot C_6H_4\cdot CO_2Na + NaCl$$

The product is then hydrolyzed with n-alkali at 20° to give the didepside,

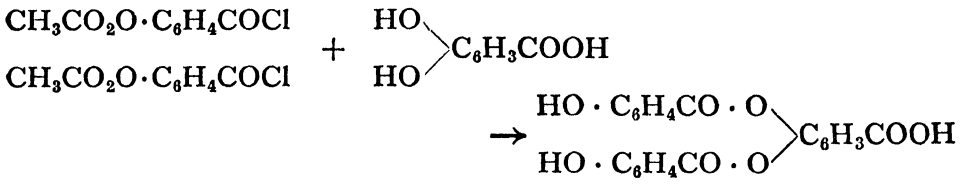
$$HO\cdot C_6H_4\cdot CO\cdot O\cdot C_6H_4COOH$$

Tri- and tetra-depsides.—These are obtained in a similar manner to the didepsides. In the case of the monophenolcarboxylic acids only one class of polydepside is possible, viz. straight-chain compounds of the type:

$$HO\cdot C_6H_4CO\ldots\ldots\ldots O\cdot C_6H_4COOH$$

* *Ber.*, 1908, **41**, 2880. † *Ber.*, 1909, **42**, 1017. ‡ *Ber.*, 1909, **42**, 216.

but with di- and tri-phenolcarboxylic acids several types are theoretically possible, e.g.



Compounds of such types are not yet known with certainty.*

The following tabulation embraces the more important polydepsides prepared by these methods:

Didepsides.	Tridepsides.	Tetradepsides.
Di-*p*-hydroxybenzoic acid.†	Di-*p*-hydroxybenzoyl-*p*-hydroxybenzoic acid. ‖	Tri-*p*-hydroxybenzoyl-*p*-hydroxybenzoic acid.‖
Di-protocatechuic acid. ‡	Vanilloyl-*p*-hydroxybenzoyl-*p*-hydroxybenzoic acid.‖	Vanilloyl-di-*p*-hydroxybenzoyl-*p*-hydroxybenzoic acid.‖
m-Digallic acid. §		
Di-β-resorcylic acid. ‡		
Vanilloyl-vanillin.‖		

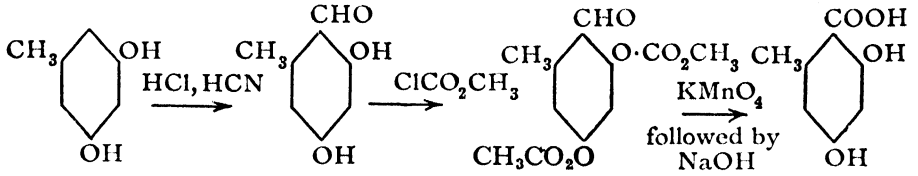
In 1918 Fischer prepared several depsides in which acetylation of the phenolic hydroxyl groups had been used instead of carbomethoxylation.† † The acetyl derivatives of the phenolic acids are easily prepared, crystallize well, and may be converted into their chlorides without difficulty. After condensation the acetyl groups may be removed by dilute alkali at zero or ammonia at ordinary temperature.

Lichen Products.—The only natural source of the depsides so far discovered is the Lichens, which are peculiar plant formations produced by the symbiosis of algæ and fungi. Many species of lichens have been used from early times in medicine, dyeing, and as food-stuffs. Two acids, lecanoric and evernic, which are found in the varieties *Roccela* and *Lecanora*, and *Evernia prunastris*, have been the subject of several investigations by Fischer and his collaborators. Orcinol (sym. methylresorcinol) is an important constituent of " litmus " lichens.

Orsellinic Acid.**—This acid has been synthesized from

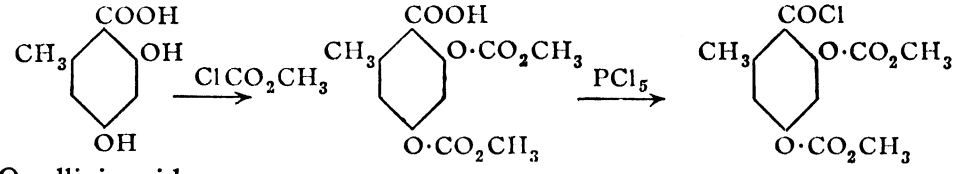
* *Ber.*, 1912, **45**, 2712. † *Ber.*, 1909, **42**, 217. ‡ *Ann.*, 1911, **384**, 238.
§ *Ber.*, 1913, **46**, 1124. ‖ *Ann.*, 1910, **372**, 63.
† † *Ber.*, 1918, **51**, 46; 1919, **52**, 809. ** *Ber.*, 1913, **46**, 886.

orcinol by Hoesch. Orcinol is converted into orcylaldehyde by Gattermann's method, and, after protecting the two hydroxyl groups by carbomethoxylation, is oxidized and subsequently hydrolyzed to give orsellinic acid:



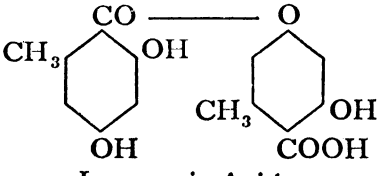
Orcinol Orcylaldehyde Orsellinic acid

Lecanoric Acid.—This acid has long been known as an ester-like anhydride of orsellinic acid. The acid has been studied and synthesized by Fischer.* For this purpose orsellinic acid was converted into its dimethylcarbonato derivative and thence into the corresponding acid chloride.



Orsellinic acid

This chloride was then coupled with orsellinic acid in aqueous acetone solution, and on hydrolysis gave a diorsellinic acid identical with lecanoric acid. Since orsellinic acid contains two hydroxyl groups, it is evident that this synthesis alone does not establish the structure of lecanoric acid. The authors have however synthesized *o*-diorsellinic acid and found it to be different from lecanoric acid, from which it is evident that in the above synthesis the acid chloride condenses with the *p*-hydroxyl group of orsellinic acid, and the constitutional formula of lecanoric acid thus becomes:

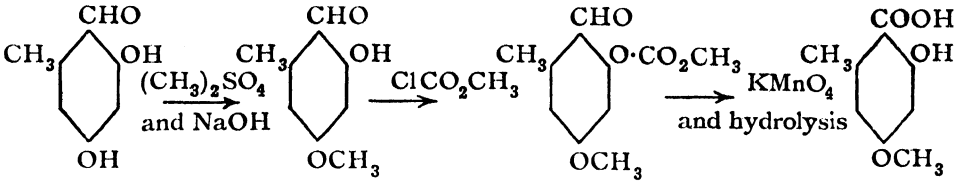


Lecanoric Acid

Everninic Acid.—This acid, together with orsellinic acid, is obtained by the hydrolysis of evernic acid with baryta. Everninic

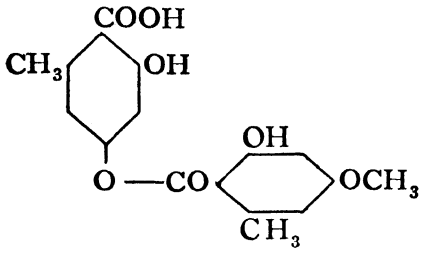
* *Ber.*, 1913, 46, 1138.

acid has been synthesized from orcylaldehyde by Hoesch as follows:



Orcylaldehyde Everninaldehyde Everninic acid

Evernic Acid.—When natural evernic acid is methylated by means of diazomethane it gives a product identical with that obtained by the methylation of lecanoric acid, viz. the methyl ester of trimethyl-lecanoric acid. Evernic acid must therefore be a monomethyl-lecanoric acid, and since on hydrolysis it gives everninic acid, the methyl group must be in the para position to the depside group.



Evernic acid

Gallotannin.—It has already been stated that Strecker had denoted gallotannin as a compound of one molecule of grape sugar with three molecules of gallic acid. For half a century there prevailed a conflict of opinion as to the presence of a glucose residue, the production of sugar on hydrolysis being denied by several chemists, and the proportions in which it was obtained by the followers of Strecker varying much amongst themselves.

Before studying the hydrolysis of gallotannin it was, of course, necessary to obtain a sample as pure as possible. For this purpose Fischer and Freudenburg * employed Chinese tannin and purified it by extraction from a weak alkaline solution with ethyl acetate. On hydrolysis with 5 per cent sulphuric acid for 70 hours it yielded 7 to 8 per cent sugar, an amount which they regarded as probably too low in view of the extended period occupied in completing the reaction. They then expressed the opinion that the principal constituent of tannin is not a glucoside, but a sugar ester comparable

* *Ber.*, 1912, **45**, 919.

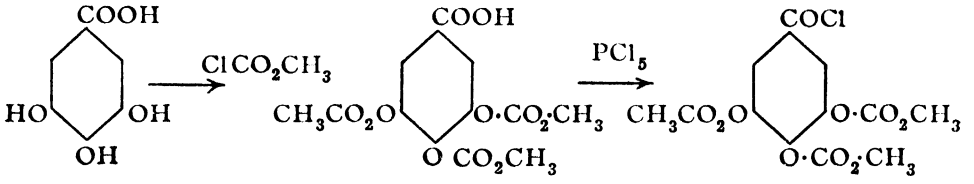
with pentabenzoylglucose, in which the acyl group is that of digallic acid. Expressed by the formula

$$C_6H_7O_6[C_6H_2(OH)_3 \cdot CO \cdot O \cdot C_6H_2(OH)_2 \cdot CO]_5$$

such a compound having a molecular weight 1700 would yield 10·6 per cent of glucose on hydrolysis.

Somewhat later Fischer and Bergmann * made use of the potassium salt of gallotannin as a method of purification—a method originally recommended by Berzelius.

Pentagalloyl-glucose.—Fischer and Freudenburg then turned their attention to the synthesis of pentagalloyl-glucose. For this purpose trimethylcarbonatogalloyl chloride was prepared from gallic acid as follows:



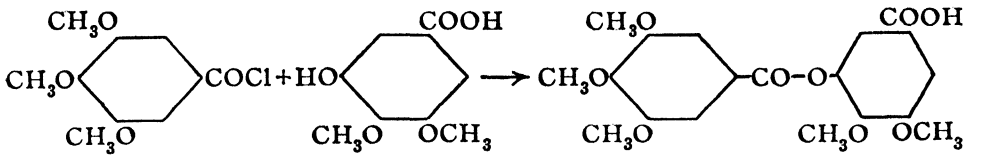
This acid chloride was then condensed with glucose in chloroform solution in the presence of quinoline to give penta (trimethylcarbonato-galloyl) glucose, which on hydrolysis with alkali in aqueous acetone gave pentagalloyl glucose. Both the α and β forms were obtained by fractional crystallization and, although not identical with gall-nut tannin, closely resembled it in amorphism, taste, solubility, optical activity, and feeble acidity. Moreover, the product precipitated gelatin and alkaloids, became gelatinous with arsenic acid, and developed a colour with ferric chloride.

Methylotannin.—In 1905 Herzog † had obtained a compound which he termed " methylotannin " by the action of diazomethane on tannin. This compound was indifferent towards alkali and therefore contained no carboxyl or hydroxyl groups. On hydrolysis it gave trimethylgallic acid and *m-p*-dimethylgallic acid. Taking this evidence, in addition to his earlier work, Fischer concluded that gallotannin probably consists of a compound of one molecule of glucose with five molecules of digallic acid. Methylotannin would then consist of one molecule of glucose with five molecules of pentamethyl-*m*-digallic acid.

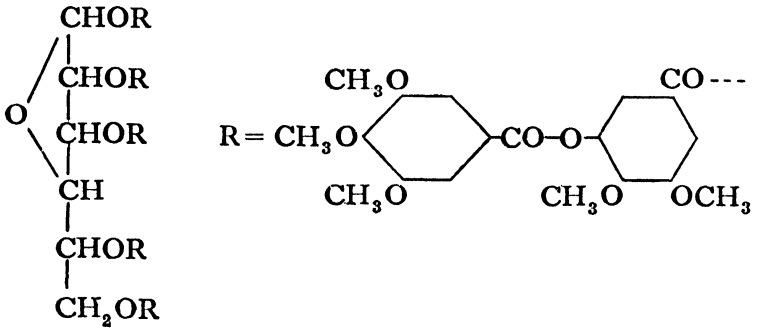
Pentamethyl-*m*-digallic acid was next synthesized by condensing

* *Ber.*, 1919, **52** [B], 829. † *Ber.*, 1905, 38, 989.

trimethylgalloyl chloride with the *m-p*-dimethyl ether of gallic acid in the presence of alkali:



The chloride of this acid was then coupled with α- and β-glucose to give penta (pentamethyl-*m*-digalloyl) glucose which, from its properties, appeared to be identical with methylotannin.



Several unsuccessful attempts were then made to prepare *p*-digallic acid *, but owing to the wandering of an acyl group, the meta acid was invariably obtained, even when the most delicate methods were used for hydrolysis.

The Active Principle of Chinese Tannin.—Valuable as the use of the methylcarbonato derivatives had proved, they did not suffice to perfect the aim in view, namely to synthesize the main principle of Chinese tannin. This was accomplished in 1918, following the observation that the corresponding acetyl compounds are superior to the methylcarbonato derivatives for depside production. In making this advance, Fischer explained that the acetylated phenolcarboxylic acids would certainly have been used much earlier had not he been misled by the statements of previous workers as to the difficulty of removing the acetyl group, which actually proceeds quite smoothly.

The chloride of penta-acetyl-*m*-digallic acid is crystalline, and with β-glucose yields the compound:

$$C_6H_7O_6[C_6H_2(O \cdot COCH_3)_3 \cdot CO \cdot O \cdot C_6H_2(O \cdot CO \cdot CH_3)_2 \cdot CO-]_5$$

This is then de-acetylated by cold aqueous caustic soda at zero,

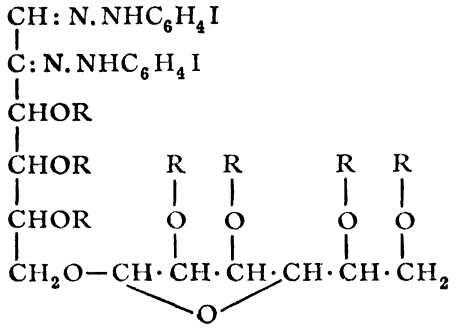
* *Ber.*, 1918, **51**, 45.

giving penta (*m*-digalloyl) β-glucose. The resemblance between this artificial tannin and the principal constituent of Chinese tannin is much closer than that offered by pentagalloylglucose.

More recently Nierenstein * has criticized Fischer's formula for gallotannin, and suggested an alternative formula in which four of the hydroxyl groups of the glucose portion of the molecule are free, but the reader is referred to the original literature for an account of these criticisms. It need hardly be pointed out that Fischer's work deals only with one particular tannin, and that the constitution of many of the tannins, and especially the catechol tannins, is still obscure.

Many of the intermediate compounds described in this chapter are substances of very high molecular weight, e.g. the M.W. of penta (pentamethyl-*m*-digalloyl) glucose is 1810. During these investigations Fischer synthesized hepta (tribenzoyl-galloyl) *p*-iodophenylmaltosazone, a freak molecule of gigantic dimensions (M.W. 4021), vastly exceeding that of any other synthetic product. †

Maltose → *p*-iodophenylmaltosazone →
(*p*-Iodophenylhydrazine) (tribenzoyl-galloyl chloride)



where $R = -CO \cdot C_6H_2(O \cdot COC_6H_5)_3$

Hepta (tribenzoyl-galloyl) *p*-iodophenylmaltosazone [$C_{220}H_{142}O_{58}N_4I_2$]

REFERENCES.

The Principles of Leather Manufacture, by Proctor (London, 1903).
Die Chemie der natürlichen Gerbstoffe, by Freudenburg (Berlin, Julius Springer).
Nekrolog auf Emil Fischer, by Hoesch (*Berichte*, 1921).

* *J. Soc. Chem. Ind.*, 1922, **41**, 29 T. † *Ber.*, 1913, **46**, 1116

CHAPTER V

Animal and Vegetable Oils, Fats, and Waxes

Introduction and Classification.—Oils, fats, and waxes may be divided broadly into two natural groups, those of animal and plant origin, and those of mineral origin. The former may again be subdivided into two groups, according as they are volatile or non-volatile. The volatile oils, which are contained mainly in the leaves, stems, and flowers of the plant, are also termed essential oils and will be dealt with in a subsequent chapter. The non-volatile oils, which are also termed " fixed " oils, are contained in the seeds and fruits of plants and are obtained by means of expression, or by extraction with solvents. Considered chemically, all fixed oils and fats are esters of glycerol with fatty acids, and are termed glycerides. The animal and vegetable waxes are also esters, but the neutral radicle contained in these is an alcohol other than glycerol and consequently no glycerol can be obtained from them.

The manufacture of glycerol, soap, and candles from natural fats, as well as the hardening of oils with the production of edible and hydrogenated oils, are too well known to require elaboration here.

During the Great War efforts were made to produce fatty acids by the oxidation of suitable hydrocarbons, while in order to conserve glycerol supplies attempts were made to prepare edible fats containing ethylene glycol and other polyhydric alcohols in place of glycerol. Considerable success was also achieved in the preparation of glycerol by the fermentation of sugar.

A very short account will be given of the chemistry of the lipins, from which it will be seen that as yet we have very little knowledge of the chemical constitution of these substances.

Occurrence.—The oils, fats, and waxes are very widely distributed in the vegetable kingdom, and they are found in especially

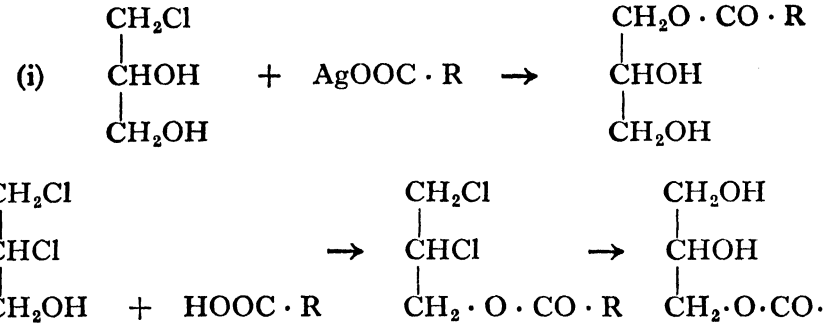
large amounts in the reproductive bodies such as spores and seeds. The commonest glycerides are those of oleic, palmitic, and stearic acids, but the glycerides of many other acids—such as, for instance, linoleic and linolanic acids in linseed oil, erucic acid in colza and rape oil, lauric acid in laural oil, myristic acid in oil of nutmeg, and ricinoleic acid in castor oil—are found in smaller quantities. The majority of vegetable fats are liquid at ordinary temperatures, but a few, such as coco-butter, are solid. The oils and fats form one of the most important food reserves of plants, and they are probably formed from carbohydrates, of which glucose, sucrose, and starch appear to be those most usually employed.

The glycerides of fatty acids occur in animals, stored in the connective tissue cells of adipose tissue, and for the most part these glycerides are esters of stearic, palmitic, and oleic acids. In certain animals the glycerides of other fatty acids occur; thus, lard contains about 10 per cent of acids of the linoleic series, while in the fat of cows' milk the esters of butyric and caproic acids occur in fair quantities, and those of the intermediate acids, caprylic, capric, lauric, and myristic acids in traces.

The glycerides which occur in nature contain, in almost all cases, three fatty acid radicles, and are thus triglycerides. These triglycerides have frequently been supposed to be each a compound of glycerol with one and the same acid, that is, to be simple triglycerides; but several mixed triglycerides, or compounds containing more than one acid united with the same molecule of glycerol, have now been separated from natural products.

The Constitution and Synthesis of the Glycerides.—The constitution of the oils and fats as triglycerides was established by the classic researches of Chevreul carried out between 1815 and 1823.

Since glycerol is a trihydric alcohol it should be possible to obtain mono-, di-, and tri-glycerides; and, further, since glycerol is both a primary and a secondary alcohol, two different mono- and di-glycerides should be obtainable from glycerol and an acid. A perusal of the literature will show that many of these compounds have been obtained. The method most commonly employed for the preparation of monoglycerides depends either on the action of glycerine chlorohydrins on the salts of fatty acids (i), or on the esterification of the fatty acid with the chlorohydrin and subsequent exchange of the halogen atoms for the hydroxyl groups (ii).



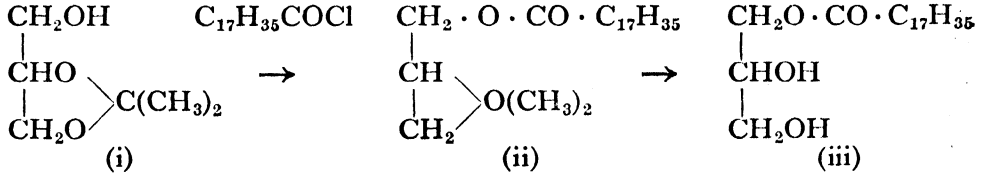
Both these methods have recently been shown to be unreliable by Fischer,* since the former method is complicated by side reactions, and no guarantee is afforded of the simple replacement of the halogen atoms by the fatty acid radicals, while in the second method the halogen atom can, in general, be only replaced under conditions which readily occasion further change.

Attention was drawn by Fischer to the fact that glycerol α-monobenzoate is rapidly converted into a mixture of glycerol and a dibenzoate when treated in ethereal solution with potassium carbonate. The process is more conveniently followed with the benzoyl derivatives of ethylene glycol in chloroform solution, whereby it may be shown that the change is balanced and attains an equilibrium in the presence of the glycol and the mono- and di-benzoates. Similarly, glycerol monoacetate is largely transformed into diacetin and glycerol. The action of the potassium carbonate appears to be definitely catalytic, since very small amounts of it suffice to accelerate the change. The phenomena are very similar to those first observed by Purdie,† who found that an exchange of alkyl radicals readily took place between simple esters and alcohols in the presence of a small amount of sodium alkyloxide. Grun ‡ has shown that this interchange of alkyl groups between fats and alcohols can take place under certain conditions even in the absence of catalysts.

Such phenomena explain to some extent the gradual change in the melting-point observed by Grun to take place when diacyl derivatives of glycerol are preserved, and also of the so-called ageing of the natural fats. This, however, is not a complete explanation of all the facts, as some of the triglycerides are known in more than one form, as, for example, tristearin which exists in two forms, melting-points 55° and 71° respectively. Grun prefers to regard these as examples of co-ordination isomerism, but this hypothesis is somewhat intangible.

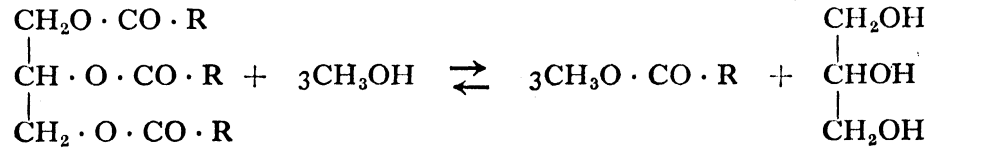
* *Ber.*, 1920, 53, 1589. † *Trans.*, 1887, 53, 391. ‡ *Ber.*, 1921, 45 [B], 273, 290.

This circumstance has rendered the synthesis of pure monoglycerides a matter of considerable difficulty. As initial material for the synthesis of monoglycerides, Fischer, Bergmann, and Barwind * adopted "acetone glycerol (i) (p. 61), the constitution of which has been definitely established by Irvine, Macdonald, and Souter.† This substance readily reacts with acid chlorides in the presence of quinoline, yielding products from which the acetone residue is easily removed by mild treatment, thus giving undoubted α-monoglycerides. For example, stearyl chloride condenses with isopropylidene glycerol in the presence of quinoline to give stearyl isopropylidene glycerol (ii). On hydrolysis with semi-normal hydrochloric acid in the presence of ether, α-monostearin (iii) is obtained.



Hydrolysis of the Oils and Fats.—The glycerides are hydrolyzed by superheated steam in a few hours, and more readily in the presence of hydrochloric acid acting as a catalyst. Sulphuric acid acts more rapidly than hydrochloric acid probably because it helps to bring the oil into a state of fine division or emulsification. Twitchell's reagent—which is an aromatic derivative of sulphuric acid obtained by dissolving oleic acid in benzene or naphthalene in oleic acid and adding strong sulphuric acid—is used very extensively for this purpose. The aromatic sulphonic acid is the catalyst, and it acts more rapidly than sulphuric acid because it is soluble in fat, fatty acid, and water. The addition of small quantities of lime or magnesia accelerates the hydrolyzing action of steam, and if similar small quantities of the alkalies which give soluble soaps be added, the acceleration is even more pronounced.

The removal of glycerol from its union with fatty acids in glycerides may be effected by alcohols containing as catalyst 1 to 2 per cent of hydrochloric acid. The following reaction takes place in the case of methyl alcohol:



* *Ber.*, 1920, 53 [B], 1589. † *Trans.*, 1915, **107**, 337.

The excess of alcohol sets the equilibrium point very much towards the right-hand side of the equation, and the presence of hydrochloric acid causes this equilibrium to be rapidly approached.

Lapworth and Pearson * have shown that glycerol can be quantitatively replaced by mannitol in fats by heating the fat with mannitol in the presence of sodium ethoxide under reduced pressure. An almost theoretical yield of glycerol is obtained in the distillate, while the residue in the distillation flask may be treated so as to obtain a synthetic mannitol fat. The maximum yield of glycerol is obtained when the proportion is two molecules of fat to three of mannitol. The nature of the fat thus obtained is not known with certainty.

Enzymes which are capable of hydrolyzing fats occur in the seeds in which vegetable oils are found, and lipase is the most widely distributed of these enzymes. It is found in the pancreatic juice, the liver and blood of animals, and in most oily seeds particularly during germination. Lipase is not only able to hydrolyze fats, but also many other esters, such as ethyl salicylate, ethyl acetate, and ethyl carbonate. There appears to be a distinct difference between the enzymes from different sources, and it has been stated that lipase may be separated into two substances, neither of which is independently capable of bringing about the hydrolysis. Lipase has a reversible action, and the fact whether it hydrolyzes or synthesizes fats is merely a question of conditions, mainly the presence or absence of water. The glycerol extract of a fat-containing seed—which extract contains the lipase—mixed with oleic acid will synthesize a fat; while the addition of water results in the hydrolysis of this fat into glycerol and fatty acid.

As early as 1855, Pelouze showed that oil seeds contain a substance which is capable of producing comparatively rapid hydrolysis of the oils contained in the seeds; but little attention was paid to this subject from a technical point of view until Constein, Hoyer, and Wartenberg,† by an extended series of experiments, showed that the ferment contained in castor seeds is capable of accelerating considerably the hydrolysis of triglycerides, provided they be completely emulsified in a slightly acid medium.

The enzymes contained in animal organisms appear to act much more slowly than those occurring in the seeds of plants.

The changes undergone by fats and oils when they become rancid are possibly initiated by enzymes that hydrolyze the glycerides, but there is, as yet, little definite information on this subject.

* *Biochem. J.*, 1919, **13**, 296. † *Ber.*, 1902, **35**, 3989.

The Waxes.—It has already been mentioned that the waxes must be considered as esters formed by the combination of mono- or di-hydric alcohols with higher fatty acids. The alcohols hitherto identified in waxes belong both to the aliphatic and cyclic series, the latter being represented by the sterols.

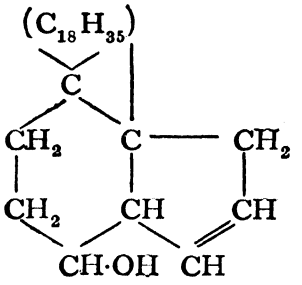
One of the best known vegetable waxes is carnauba wax from a South American palm (*Copernicia cerifera*). It is a hard brittle compound, the constitution of which is unknown. This wax contains ceryl alcohol, $C_{26}H_{53}OH$, myriscyl alcohol, $C_{30}H_{61}OH$, and two acids, cerotic acid, $C_{26}H_{52}O_2$, and carnaubic acid, $C_{24}H_{48}O_2$, together with a hydroxy acid. Candelilla wax is obtained from the stem of a leafless plant (*Pedilanthus pavonis*), growing chiefly in Mexico. Its composition is unknown, but a hydrocarbon, hentriacontane, $C_{30}H_{62}$, has been isolated from it.

Animal waxes are obtained from a great variety of sources and have little in common with those from vegetable sources except the absence of glycerides. The following are some of the more important animal waxes: wool wax, wool fat, or lanoline, which is rich in cholesterol; bees-wax, which consists principally of myricyl palmitate and cerotic acid; spermaceti, which is obtained as a solid precipitate from the head oil of the sperm and bottle-nosed whale, and consists almost entirely of cetin and cetyl palmitate; and insect or Chinese wax, which consists mainly of ceryl cerotate.

The Sterols : Cholesterol and Phytosterol.—In addition to the trihydric alcohol glycerol, all fats contain a small quantity of the monohydric cyclic alcohols—cholesterol and phytosterol, which form what is known as the " unsaponifiable residue " of fats. These substances may be isolated from fats by treating an ethereal solution with alcoholic sodium ethoxide, when saponification takes place and the soap separates. The filtrate then contains glycerol and the sterols. All animal fats contain cholesterol, while vegetable fats contain either phytosterol itself or a closely allied substance belonging to the group of sterols. Cholesterol is frequently met with in the animal organism; thus, biliary calculi are almost wholly composed of cholesterol, while its presence has been further confirmed in human bile, blood, brain, epidermis, in milk, and in the yolks of egg. It may be conveniently prepared by evaporating to dryness the ethereal extract of gall-stones.

Cholesterol, $C_{27}H_{45}OH$, has been the subject of prolonged investigation especially by Windaus. Its constitution is as yet unknown, but it appears to be a polycyclic, hydroaromatic, secondary

alcohol containing four reduced rings. Windaus * considers that its constitution has been established to the extent indicated in the expression:



The term phytosterol was at one time employed to designate a definite chemical individual of the formula $C_{27}H_{45}OH$, but is now used as a generic term to include a number of substances having certain properties in common. Windaus and Hauth † showed that the substance obtained from calabar beans and commonly known as phytosterol was in reality a mixture of two substances, sitosterol, of the formula $C_{27}H_{45}OH$, and stigmasterol, $C_{30}H_{47}OH$—an observation which has been confirmed by Salway.‡ Sitosterol, the "cholesterol of plants", is widely disseminated in the vegetable kingdom, and occurs in all seeds and fruits. It differs from cholesterol in crystalline form, melting-point, magnitude of optical rotation, and chemical constitution, the latter being as yet unknown.

Other sterols which have been described include, isocholesterol, $C_{27}H_{46}O$, brassicasterol, and stigmasterol.

The Preparation of Fatty Acids from Hydrocarbons.—During the late war strenuous efforts were made by the Central Powers to use paraffin wax as an initial material for the production of fatty acids and their esters in order to overcome the shortage of natural fats. The usual method was to heat the hydrocarbons of high molecular weight with oxygen or air, generally under pressure, in the presence of a catalyst. Thus, in the presence of manganese compounds, § C. Kelber converted a paraffin wax (m.p. 50°), by the action of a stream of oxygen at 150°, into a mass of which more than 35 per cent consisted of fatty acids insoluble in water, and about 25 per cent of the lower fatty acids (up to capric acid, $C_{10}H_{20}O_2$).

H. H. Franck || used up to 5 per cent of various compounds

* *Ber.*, 1919, **52** [B], 162. † *Ber.*, 1906, **39**, 4378; 1907, **40**, 3681.
‡ *Trans.*, 1911, **99**, 2154. § *Ber.*, 1920, **53** [B], 66, 1567.
|| *Chem. Zeit.*, 1920, **44**, 309.

of lead, mercury, vanadium, and chromium, and, working at 150° in an autoclave filled with oxygen, obtained from a paraffin of low melting-point 40 per cent of fatty acids of higher, and 57 per cent of acids of lower molecular weight. A mixture of the acids so obtained was esterfied with ethylene glycol, and yielded an edible fat said to resemble coco-nut oil. F. Fischer and W. Schneider * employed a steel autoclave, and conducted the reaction at 170° in the presence of sodium carbonate, the mixture being stirred by pumping in compressed air. In this way they obtained a yield of 90 per cent of fatty acids from crude paraffin, and these authors are of the opinion that iron, copper, and manganese have equal catalytic effects.

A. Grun † has studied these reactions in more detail, and has shown that the results are dependent on many factors as yet little understood. In the absence of water the anhydrides of the higher fatty acids are formed, and in every case the neutral products contain ketones, such as stearone. The acids formed all appear to have a straight chain structure, while, according to Fischer and Schneider, the acids containing an uneven number of carbon atoms are formed in greater quantity than those with an even number. The latter type are those commonly derived from natural fats.

Schaarschmidt and Thiele ‡ have chlorinated paraffin at 160°, and removed the hydrogen chloride either by treatment with alkali or by simply heating at about 300°. The resulting mixture of olefines was then oxidized, preferably by ozone, and under the best conditions a yield of 60 per cent of the higher fatty acids was obtained. Similar results were obtained by Granacher,§ who oxidized heated paraffin wax by a current of air containing 2 per cent of nitrogen peroxide. At 150° the process requires about four days. When *n*-undecane is treated in the same manner, nonoic is the highest acid formed, and this is only obtained in small quantities. This method is therefore unsuitable for the degradation of hydrocarbons, but it indicates that the higher paraffins in nature probably consist only to a small extent of normal hydrocarbons.

Fermentation Glycerol.—As early as 1858 Pasteur observed the formation of small quantities of glycerol during the course of alcoholic fermentation. During the war, this circumstance assumed enormous importance in Germany, for it made possible the production of glycerol from sugar on an industrial scale. It was dis-

* *Ber.*, 1920, 53 [B], 922. † *Ibid.*, 987. ‡ *Ber.*, 1920, 53 [B], 2128.
§ *Helv. Chim. Acta*, 1920, 3, 721.

covered that, under special conditions, the ordinary yield of glycerol of about 3 per cent can be increased at least tenfold.

The essential feature of the industrial process, which has been described by K. Schweizer,* and by W. Constein and K. Ludecke,† is the employment of sugar solutions containing large quantities of sodium sulphite. Crude sugar, or even molasses, may be used, and neither the race of yeast nor the temperature appears to have much influence on the yield of glycerol. The monthly output in Germany finally amounted to 1000 tons, 100 parts of sugar yielding 20 parts of purified glycerol, 27 of alcohol, and 3 of acetaldehyde.

The process is based on the work of Neuberg and his pupils, and this chemist has furnished a theoretical explanation in a paper‡ which can only be briefly summarized here. In 1913 Neuberg and Kerb § put forward the hypothesis that dextrose, by loss of two molecules of water, furnishes the aldol of methylglyoxal, $C_6H_8O_4$, which breaks down to two molecules of this keto-aldehyde, $C_3H_4O_2$, one of which is reduced to glycerol, while the other is oxidized to pyruvic acid:

$$\begin{matrix} CH_2:C(OH)CHO + H_2O \\ CH_2:C(OH)CHO \end{matrix} + \begin{matrix} H_2 \\ \| \\ O \end{matrix} = \begin{matrix} CH_2(OH)\cdot CH(OH)\cdot CH_2OH \\ + \\ CH_3CO\cdot COOH \end{matrix}$$

The pyruvic acid is decarboxylated by carboxylase to acetaldehyde,

$$CH_3\cdot CO\cdot COOH = CO_2 + CH_3CHO$$

and the latter is reduced to alcohol, while from a further molecule of methylglyoxal, pyruvic acid is regenerated:

$$\begin{matrix} CH_3\cdot CO\cdot CHO \\ CH_3\cdot CHO \end{matrix} + \begin{matrix} O \\ \| \\ H_2 \end{matrix} = \begin{matrix} CH_3CO\cdot COOH \\ + \\ CH_3CH_2OH \end{matrix}$$

Hence methylglyoxal and pyruvic acid would be intermediate stages, and glycerol and acetaldehyde necessarily by-products. As a matter of fact, the latter are always present during alcoholic fermentation, and the circumstance that the only known form of methylglyoxal does not ferment is not a fatal objection, since it is probably the most stable of the many possible forms.

It was next found || that slightly alkaline salts do not suppress the fermentation, but increase the yield of the by-products at the expense of the main products; and then it was shown ** that by

* *Helv. Chem. Acta*, 1919, **2**, 167. † *Ber.*, 1919, **52** [B], 1385.
‡ *Ibid.*, 1677. § *Biochem. Zeitsch.*, 1913, **58**, 158.
|| *Biochem. Zeitsch.*, 1916, **78**, 238. ** *Ibid.*, 1918, **89**, 365.

the use of sodium sulphite the acetaldehyde may be fixed in a yield of 70 per cent of the theoretical as the additive compound $CH_3 \cdot CH(OH) \cdot O \cdot SO_2Na$. The similar additive compound of pyruvic acid undergoes decarboxylation. As the acetaldehyde is now no longer reduced, the " hydrogen of fermentation " is used up in forming more glycerol. Since the aldehyde-sulphite compound dissociates, its yield, and that of the glycerol, should depend on the concentration of the sodium sulphite employed, but not be proportional to it (mass action). The theory further demands that glycerol and acetaldehyde should be formed in molecular proportions. Both these postulates are fulfilled; thus, from 100 gm. of dextrose and varying amounts of sulphite, the following yields were obtained:

Sodium Sulphite Used.	Acetaldehyde Grammes.	Glycerol Grammes.
33	11·90	23·37
50	12·52	24·86
75	13·89	27·61
150	18·65	36·90

The molecular ratio acetaldehyde : glycerol is therefore 0·94 to 0·95 instead of 1. The highest yield of glycerol corresponds with 35·06 per cent of hexose, or 70 per cent of the portion which could furnish glycerol. For a 100 per cent conversion, the fermentation would have to proceed completely according to the equation:

$$C_6H_{12}O_6 + Na_2SO_3 + H_2O = C_3H_8O_3 + CH_3CH(OH)OSO_2Na + NaHCO_3$$

The shortage of 30 per cent is due to unsuppressed dissociation of aldehyde sulphite. With the same relative quantities of sugar and sulphite in dilute solution, the dissociation is much greater and the yield of glycerol falls off considerably.

Still more recently Neuberg and Reinfurth state that insoluble calcium sulphite suspended in the fermenting solution has advantages over the sodium salt.*

The Lipins.—When animal and vegetable tissues are extracted with ether or with certain other organic solvents, the extract is composed of a heterogeneous collection of substances which include (1) neutral fat and fatty acid; (2) substances having no relation to the fats, such as cholesterol and certain pigments; (3) substances containing fatty acids, nitrogen, and phosphorus, known as phos-

* For a summary of the methods by which the normal course of fermentation has been modified to produce glycerol, see Schweizer (*Chim. et Industrie*, 1921, **6**, 149).

phatides; and (4) cerebrosides, which are substances containing fatty acids, nitrogen, and a carbohydrate, but no phosphorus.

The last two groups of fat-like bodies are often termed lipoids, but as these substances are difficult to separate and to obtain in a pure state, much confusion prevails regarding their number and chemical properties. The term " lipoid " has been used in such a vague and unsatisfactory sense—sometimes even including the neutral fats—that it is better to consider the phosphatides and cerebrosides together as lipins. The lipins can then be defined as substances of a fat-like nature yielding on hydrolysis fatty acids or derivatives of fatty acids and containing in their molecule either nitrogen or nitrogen and phosphorus.

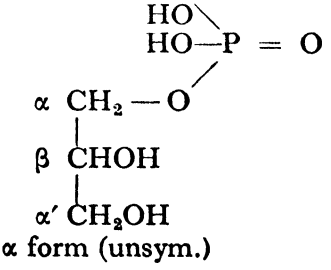
The Phosphatides: Lecithin.—The phosphatides are of plastic consistence and have distinctly fat-like properties. They occur abundantly in eggs, brain, heart, muscle, liver, and other organs, and appear to be present in every animal and vegetable cell so far investigated. On hydrolysis the phosphatides yield phosphoric acid or glycerophosphoric acid, fatty acids, and basic bodies such as choline and amino-ethyl alcohol (p. 184).

The best known phosphatides are lecithin and the closely related substance, kephalin. Both these substances contain fatty acids of the unsaturated series and are therefore very liable to oxidation. On this account their properties and solubilities soon alter on exposure to light and air, so that their extraction and isolation in an unchanged state is attended with great difficulty.

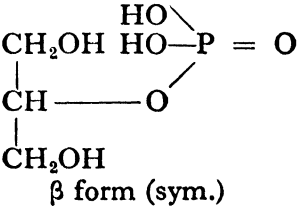
Since lecithin yields a fatty acid, glycerol, phosphoric acid, and choline on hydrolysis, we may provisionally write its structure:

$CH_2O \cdot CO \cdot R$
|
$CHO \cdot CO \cdot R$
|
CH_2 — phosphoric acid radical—choline radical

But it is obvious that glycerophosphoric acid may exist in two isomeric forms, namely an α and a β form.



α form (unsym.)



β form (sym.)

The α form contains an asymmetric carbon atom, and therefore should be capable of resolution. Willstätter and Ludecke * have shown that the glycerophosphoric acid of lecithin is optically active. This shows that the α form is present, but it does not exclude the possibility of the β form being present as well; indeed, Tutin and Hann † have adduced evidence that both forms are present.

The problem of the constitution of lecithin is by no means settled. There is some evidence that two substances containing two different bases are present, and the nature of the union of these bases with α- and β-glycerophosphoric acid is quite unsettled. The nature of the fatty acid has not been determined, and it would appear that homologues of lecithin containing different fatty acids exist.

In kephalin the base is amino-ethyl alcohol.

The Cerebrosides.—These compounds contain no phosphorus. On hydrolysis they give galactose, a fatty acid, and a base—sphingosine. Up to the present only two cerebrosides have been described—phrenosin and kerasin—and both are obtained from brain.

These two compounds would appear to be identical except in so far as the former contains phrenosinic acid ($C_{25}H_{50}O_3$) and the latter, lignoceric acid ($C_{24}H_{48}O_2$). The structure of sphingosine as well as these two acids is unknown.

Rosenheim ‡ has suggested constitutional formulæ for these cerebrosides, but no finality has yet been reached.

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* *Ber.*, 1904, **37**, 3753. † *Trans.*, 1906, **89**, 1749.
‡ *Biochem. J.*, 1916, **10**, 142.

CHAPTER VI

The Terpenes and their Derivatives

The terpenes embrace a large number of hydrocarbons of the empirical formula C_5H_8, and four main groups are recognized:

Hemiterpenes, C_5H_8.
Terpenes proper, $C_{10}H_{16}$.
Sesquiterpenes, $C_{15}H_{24}$.
Polyterpenes, $(C_5H_8)_n$.

The terpenes proper and the sesquiterpenes form the most important constituents of the ethereal oils, and they are widely distributed in plants, especially in the coniferæ and citrus species.

To O. Wallach belongs the credit of having elevated the methods of investigation of the terpenes to such a degree that the recognition and separation of the several terpene hydrocarbons have become relatively simple operations.

From the terpenes a large number of alcohols and ketones of the general composition $C_{10}H_{16}O$, $C_{10}H_{18}O$, and $C_{10}H_{20}O$ are derived. These compounds are sometimes collectively termed " camphors ", since the commercially important, common or Japanese camphor is one of them.

The study of the terpenes and their derivatives has attracted a large number of chemists, among whom Baeyer, Perkin junior, Semmler, Wagner, and especially Wallach are noteworthy. In many cases, such as dipentene, terpinene, sylvestrene, &c., complete syntheses have been carried out, while in other cases, such as pinene, camphene, &c., at least a partial synthesis has been effected. The isolation and purification of the camphors is usually much easier than that of the terpenes, since the former often crystallize well or

form characteristic crystalline derivatives. Here also the elucidation of the constitution has been followed by numerous total syntheses, e.g. camphor and menthone, while other synthetic terpenes and camphors have been obtained which have not yet been found in nature.

Of the numerous hydrocarbons of the formula C_5H_8, isoprene stands in an especially close relationship to the terpenes. Isoprene occurs in the oil obtained by the dry distillation of caoutchouc. Williams, Bouchardat, Tilden, and more recently Harries, have published investigations concerning this hydrocarbon, while its constitutional formula, $CH_2 : C(CH_3) \cdot CH : CH_2$, was established by its synthesis by Euler. Of recent years isoprene has received considerable attention in connection with the problem of synthetic rubber.*

Properties of the Terpenes, $C_{10}H_{16}$.—With the exception of camphene, which is a solid, the terpenes, when pure, are colourless, strongly refractive liquids which boil without decomposition. They have a pleasant odour, are volatile with steam, and many are optically active.

The terpenes polymerize very easily, and many, such as α-pinene and β-phellandrene, are oxidized with resinification on exposure to the air. Acids transform many terpenes into isomeric analogues. Nitrosyl chloride frequently gives well defined terpene nitrosochlorides, while many terpenes react with nitrogen peroxide to give nitrosates, $C_{10}H_{16}(NO)O \cdot NO_2$, and with N_2O_3 to give nitrosites, $C_{10}H_{16}(NO)ONO$, or pseudo-nitrosites, $C_{10}H_{16}(NO)NO_2$. By the action of ozone the terpenes yield ozonides, while, with dilute potassium permanganate, they give glycols. All these reactions have been extensively employed in the determination of the constitution of the terpenes.

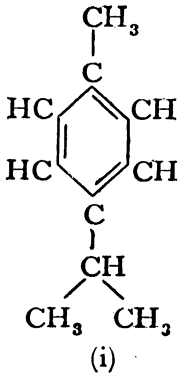
Classification.—In most cases the terpenes and camphors are designated by names derived from the plants in which they were first observed or which contain them most abundantly. Since many terpenes, formerly considered as single substances, have been found to be mixtures, the terpenes isolated from them have been distinguished from each other by prefixing Greek letters, e.g. α-, β-, and γ-terpinene.

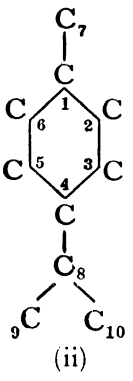
In this book some of the more important terpenes and the alcohols and ketones derived from them will be briefly considered in the following groups:

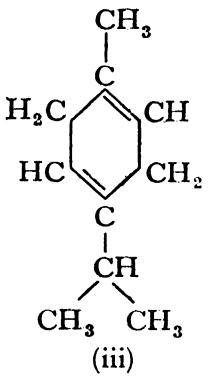
* *Rubber*, by B. D. Porritt (London, 1913).

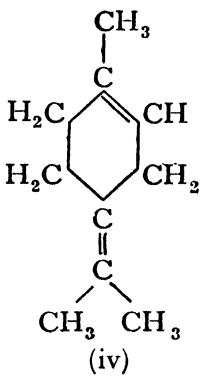
A. Olefinic terpenes, or the terpenogen group.
B. Monocyclic terpenes or menthadienes.
C. Dicyclic terpenes:
   1. The Sabinane Group.
   2. The Carane Group.
   3. The Pinane Group.
   4. The Camphane Group.

In accordance with a suggestion first made by Wagner the cyclic terpenes may be regarded as containing the same carbon skeleton as *p*-cymene (i), and Wagner designates hexahydrocymene as "menthane", the tetrahydrocymenes as "menthenes", and the dihydrocymenes or terpenes as "menthadienes". In order to indicate the constitution of the dihydrocymenes the carbon atoms are numbered according to scheme (ii), whence it follows that Δ : 1 : 4-menthadiene * and Δ : 1 : 4 : (8)-menthadiene would be represented by formulæ (iii) and (iv) respectively:


(i)


(ii)


(iii)

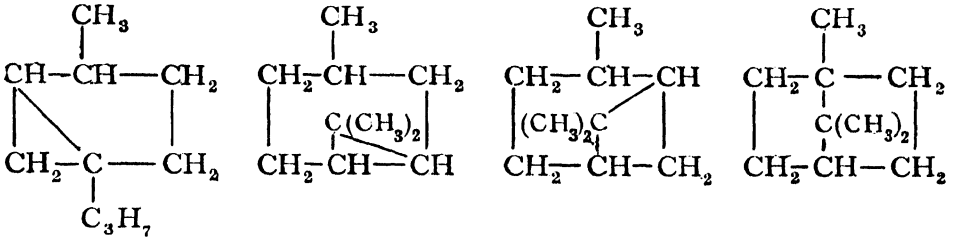

(iv)

Compounds of this class are naturally able to combine with two molecules of halogen acid, halogen, &c.

The terpenes of the dicyclic group are distinguished from those of the monocyclic terpenes by the fact that they can only add two univalent atoms or atomic groups. They therefore contain two carbon rings. Like the monocyclic terpenes, they are closely related to *p*-cymene, and can usually be converted into this compound with facility. Their dihydro compounds are derived from hexahydrocymene either by the union of two carbon atoms in the *m*-position towards each other, by a diagonal linkage—with the formation of a fused trimethylene and pentamethylene ring—which

* The position of the double linkages is indicated by the use of Δ in conjunction with the numbered position of the ten carbon atoms. The bracketed numbers indicate the second carbon attachment of the double bond outside the nucleus.

gives the sabinane group, or by the union of the tertiary carbon atom of the isopropyl group is joined with a second carbon atom of the hexamethylene ring. According as this linkage occurs in the *o*, *m*, or *p* position we obtain the fundamental hydrocarbons of the carane, pinane, and camphane groups:



Sabinane Carane Pinane Camphane

While these nuclear and bridge linkages are stable as regards the usual addition reactions, and are thus clearly distinguished from double linkages, they are broken with extraordinary facility by the action of heat and especially by hydrating agents, giving rise to derivatives of the monocyclic terpenes. It follows that the terpenes derived from them will contain one double bond only and may be described as sabinene, carene, pinene, and camphene. It is also evident that the possibilities of isomerism are more restricted than in the case of the monocyclic terpenes.

THE OLEFINIC TERPENES AND THEIR DERIVATIVES

The olefinic terpenes and their derivatives embrace a series of aliphatic compounds of the formulæ $C_{10}H_{16}$, $C_{10}H_{16}O$, and $C_{10}H_{18}O$, which were first described by Tiemann and Semmler. These open-chain terpenes bear a very close relationship to the cyclic terpenes and are easily transformed into them.

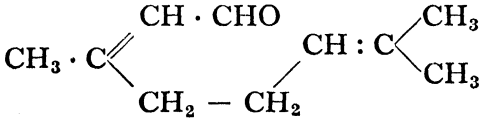
The identification of these compounds as the principal odorous constituents of many essential oils has been made in comparatively recent years. Beyond the observation of Oppenheim and Pfaff in 1874 that the oil from *Andropogon schœnanthus* gives cymol on reduction, little progress was made until 1890, when Schimmel's *Berichte* announced the fact that the aldehyde citral is the principal odorous constituent of lemon oil. In the same year Dodge announced the presence of citronellal in citronella oil, while almost at the same time Semmler isolated geraniol from oil of geranium and made some progress in the determination of its constitution.

Since this time the presence of the olefinic terpenes and their derivatives has been observed in a number of essential oils. The more important compounds of this class are tabulated opposite.

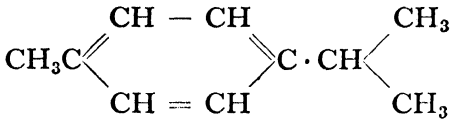
This tabulation shows that these compounds exhibit among themselves a certain similarity in structure. They contain ten carbon atoms, which are arranged in such a way that six of them form a straight chain, three of them form an unsaturated isopropyl group attached to one end of the chain, and the tenth forms a methyl group at the fourth carbon atom from the end of the chain. The grouping may therefore be considered as resembling that of a monocyclic terpene in which the ring has been ruptured.

It has already been stated that Semmler * had observed the relationship of geraniol to cymene, and that by the action of potassium hydrogen sulphate on geraniol or citral, *p*-cymene had been obtained. The relation of citral to *p*-cymene is made clear by the following formulæ:

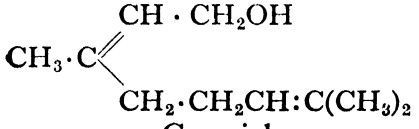
Citral,



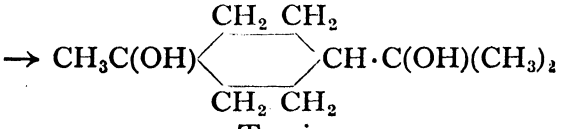
p-Cymene,



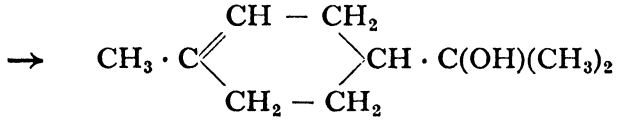
Bertram and Walbaum † showed that by dehydration of geraniol, or still better linalol, dipentene and terpinene are formed, while Stephen‡ observed that by the action of formic acid on both geraniol and linalol, terpineol is obtained. In these reactions linalol is probably first transformed into the isomeric geraniol, and, by the removal and subsequent addition of water, ring formation occurs with the production of terpin, followed by that of dipentene and terpinene.



Geraniol



Terpin



Terpineol

* *Ber.*, 1890, **23**, 1098, 2965, 3556; 1891, **24**, 201, 682.
† *J. prakt. Chem.*, 1892, **45** [2], 590. ‡ *Ibid.*, 1898, **58** [2], 109; **60**, 244.

Since this time the presence of the olefinic terpenes and their derivatives has been observed in a number of essential oils. The more important compounds of this class are tabulated opposite.

This tabulation shows that these compounds exhibit among themselves a certain similarity in structure. They contain ten carbon atoms, which are arranged in such a way that six of them form a straight chain, three of them form an unsaturated isopropyl group attached to one end of the chain, and the tenth forms a methyl group at the fourth carbon atom from the end of the chain. The grouping may therefore be considered as resembling that of a monocyclic terpene in which the ring has been ruptured.

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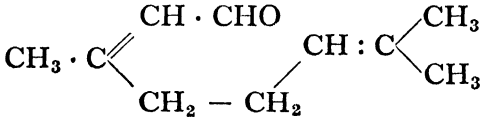
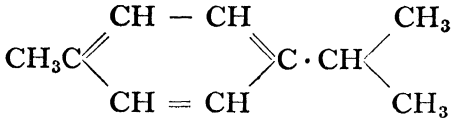
Citral,



p-Cymene,



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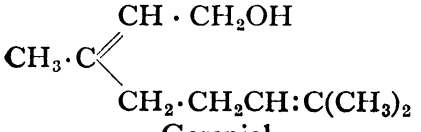
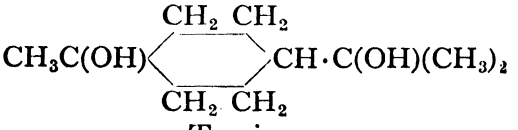
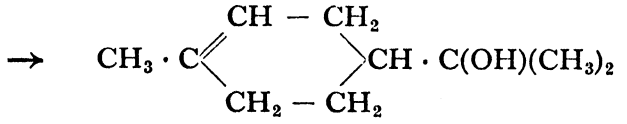


Geraniol

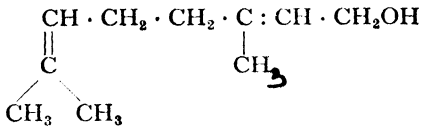
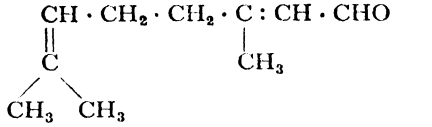
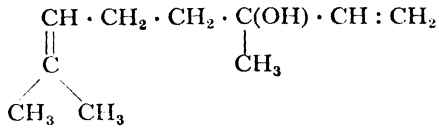
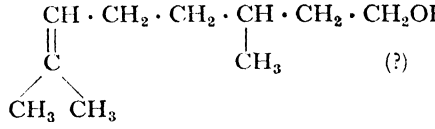
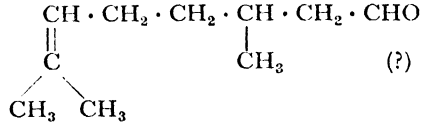
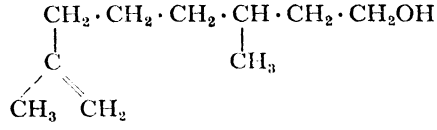
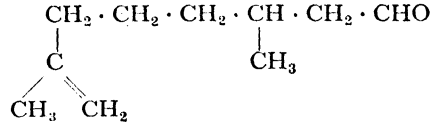


Terpin



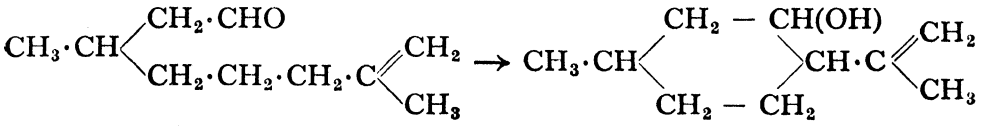
Terpineol

* *Ber.*, 1890, **23**, 1098, 2965, 3556; 1891, **24**, 201, 682.
† *J. prakt. Chem.*, 1892, **45** [2], 590. ‡ *Ibid.*, 1898, **58** [2], 109; **60**, 244.

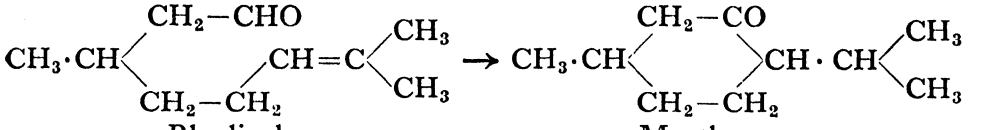
Name.	Probable Constitution.	Occurrence.
Geraniol and nerol.		*Geraniol* in geranium oil from *Andropogon schœnanthus*. ,, ,, Oil of citronella from *Andropogon nardus*. ,, ,, German and Turkish rose oils, &c. *Nerol* in neroli and petit-grain oil.
Citral and neral.		Oil of lemon-grass from *Andropogon citratus*. Oils of lemon, *Citrus limonum*. *Eucalyptus staigeriana*.
Linalol.		Oil of linaloe, oil of neroli. Oil of bergamot from *Citrus Bergamia*. Oil of lemon, petit-grain, spike lavender, ylang-ylang, &c.
Rhodinol.	 (?)	Lævo form in geranium and rose oil.
Rhodinal.	 (?)	A synthetic product.
Citronellol.		Bulgarian and German rose oil. Oils of geranium from *Pelargonium odoratissimum*, &c.
Citronellal.		Citronella oil from *Andropogon nardus*. Certain eucalyptus oils. Lemon-grass oil, oil of mandarin orange, &c.

[*Facing p.* 110

Further examples of the formation of monocyclic terpenes from olefinic terpenes are afforded by citronellal and rhodinal, which, according to Tiemann and Schmidt, are transformed by acetic anhydride into iso-pulegol and menthone respectively:



Citronellal — Iso-pulegol

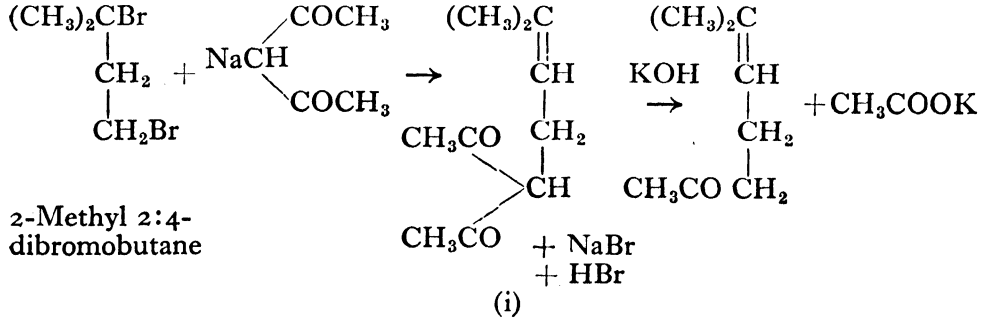


Rhodinal — Menthone

The reverse of this process takes place on exposing an aqueous alcoholic solution of menthone to bright sunlight, when the ring is opened and an unsaturated aldehyde, similar to citronellal but of lower boiling-point, is obtained.*

Before briefly describing the synthesis of the olefinic terpenes and their derivatives it is advisable to consider that of methylheptenone, since the constitutions of many of these compounds as well as their synthesis have been derived from this olefinic ketone.

Methylheptenone, $(CH_3)_2C:CH\cdot CH_2\cdot CH_2\cdot CO\cdot CH_3$, was first obtained in small quantities from natural linaloa oil by Barbier and Bouveault,† while Tiemann‡ found that it was present in lemongrass oil to the extent of 1 to 3 per cent. Methylheptenone has been synthesized by several methods, of which that of Barbier and Bouveault§ may be described. In the first place 2-methyl-2 : 4-dibromobutane is condensed with the sodium derivative of acetylacetone. This gives the unsaturated deketone (i) which can then be broken down by strong alkali into methylheptenone and acetic acid.

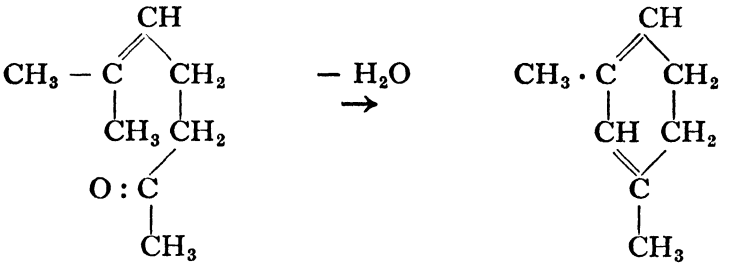


2-Methyl 2:4-dibromobutane

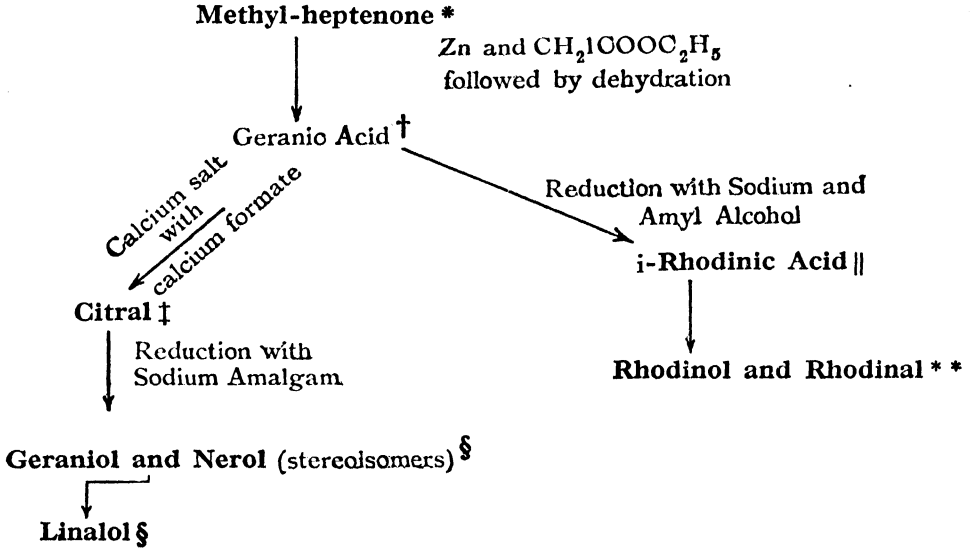
(i)

* Ciamician and Silber, *Ber.*, 1907, **40**, 2421. † *C. r.*, 1895, **121**, 168.
‡ *Ber.*, 1899, **32**, 830. § *C. r.*, 1896, **122**, 393.

When shaken with 75 per cent sulphuric acid, methylheptenone loses water and gives dihydro-*m*-xylene.



The preparation of several of the olefinic terpenes and their derivatives from methylheptenone may be illustrated by the following tabulation:



Geraniol, Nerol, and Linalol.—It has already been shown that when citral in alcoholic solution is reduced by sodium-amalgam in the presence of dilute acetic acid, geraniol and nerol are obtained.

* It is interesting to recall the fact that Barbier's attempt to convert methylheptenone into dimethylheptenol, $(CH_3)_2C:CH\cdot CH_2\cdot CH_2\cdot C(OH)(CH_3)_2$ (*C. r.*, 1899, **128**, 110), led to the discovery of the magnesium alkyl and aryl halides. Methylheptenone was allowed to react with magnesium and methyl iodide in the presence of dry ether, since Barbier had found that the zinc alkyls were unsuitable. This suggested that magnesium had reacted to form magnesium methyl-iodide, and the possibilities of this reaction were studied by Barbier's pupil, Victor Grignard. These organo-magnesium compounds are now generally designated Grignard Reagents, but would perhaps be more correctly termed Barbier-Grignard Reagents.

† Barbier and Bouveault, *C. r.*, 1896, **122**, 398; Tiemann, *Ber.*, 1898, **31**, 325.
‡ Tiemann, *loc. cit.* § Tiemann, *loc. cit.*
|| Bouveault and Gourmand, *C. r.*, 1904, **138**, 1699. ** Tiemann, *Ber.*, 1898, **31**, 2901.

These terpene-alcohols appear to be structurally identical, and are probably stereoisomerides of the formula *

$$(CH_3)_2C:CH\cdot CH_2\cdot CH_2\cdot \overset{\displaystyle CH_3}{\overset{|}{C}}:CH\cdot CH_2OH$$

When treated with acetic acid containing 1 to 2 per cent sulphuric acid both give terpineol, but the reaction is much quicker in the case of nerol, from which it is concluded that the groups which unite to form the terpineol ring are much closer together in the case of nerol than in that of geraniol:

$$\begin{array}{rl} & H-C-CH_2OH \\ & \quad\| \\ (CH_3)_2C:CH\cdot CH_2\cdot CH_2- & C-CH_3 \qquad \text{Geraniol} \end{array}$$

$$\begin{array}{rl} & HO\cdot CH_2-C-H \\ & \quad\| \\ (CH_3)_2C:CH\cdot CH_2\cdot CH_2- & C-CH_3 \qquad \text{Nerol} \end{array}$$

Linalol occurs in both dextro and lævo forms, and therefore at least one asymmetric carbon atom is present in the molecule. This and other facts are best explained on the assumption that linalol has the formula:

$$(CH_3)_2C:CH\cdot CH_2\cdot CH_2\cdot \overset{\displaystyle OH}{\overset{|}{\underset{\displaystyle CH_3}{\underset{|}{C}}}}\cdot CH:CH_2$$

but this constitution has not yet been well established.

Citronellal.—The determination of the constitution of this aldehyde may be briefly considered in order to illustrate the difficulties attendant upon this type of investigation. In 1896 Tiemann and Schmidt † obtained acetone and β-methyladipic acid by the oxidation of citronellal, and concluded—quite legitimately from their experimental results—that the constitution of citronellal was correctly represented by the formula:

$$(CH_3)_2C:CH\cdot CH_2\cdot CH_2\cdot CH(CH_3)CH_2CHO$$

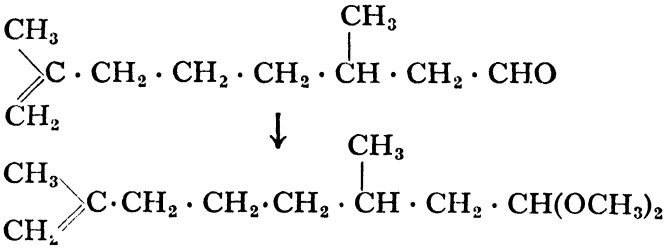
which on oxidation yields:

$$(CH_3)_2CO + HOOC\cdot CH_2CH_2CH(CH_3)CH_2COOH$$

In 1901, however, Harries and Schauwecker ‡ prepared the dimethylacetal of citronellal, which on oxidation with permanganate gave acetone and the half aldehyde of β-methyladipic acid, whereas further oxidation with chromic acid gave an oxydialdehyde and

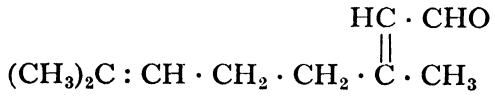
* Zeitschel, *Ber.*, 1906, **39**, 1780. † *Ber.*, 1896, **29**, 903; 1897, **30**, 22, 33.
‡ *Ber.*, 1901, **34**, 1498, 2981.

finally a keto-aldehyde, $CH_3CO{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2CH(CH_3)CH_2CHO$. These results are best explained by the following formulæ:

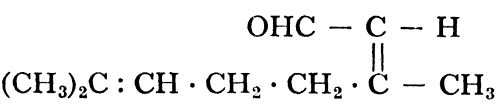


If this is so, then in Tiemann and Schmidt's experiments the double bond moved from the ultimate to the penultimate carbon atom.

Citral.—In 1907 Harries and Himmelmann* obtained evidence to show that citral exists in two geometrically isomeric forms:



Citral *a*.



Citral *b*.

The preparation of α- and β-ionone from citral will be described later.

Rhodinol and Rhodinal.—The constitution of these two compounds is by no means definitely settled as yet, and the literature contains many contradictory statements.

B.-THE MONOCYCLIC TERPENES ($C_{10}H_{16}$)

Limonene.—This terpene is known in three modifications, *d*-limonene, *l*-limonene, and (*dl*)-limonene or dipentene. Together with pinene, *dextro*limonene is among the most widely distributed terpenes. It is present in lemon oil, oil of bergamot, oil of dill, and several other natural essential oils. *Lævo*limonene occurs in oil of pine needles, oil of fir, and oil of peppermint. Dipentene, which may be obtained by mixing the two isomerides, or by racemization of either at 270°, occurs naturally in oil of cinea and is also formed by heating pinene or camphene. Dipentene is thus present in Swedish oil of turpentine, which has been prepared by distilling the natural product at a high temperature.

The limonenes combine with bromine to form tetrabromides, $C_{10}H_{16}Br_4$, which are stable crystalline substances, while nitroso-

* *Ber.*, 1907, **40**, 2823.

chlorides of the general formula $C_{10}H_{16}NOCl$ are formed by the action of nitrosyl chloride. The nitrosochloride of the inactive modification is converted into inactive carvoxime by the action of alcoholic potash. Carvone* is a ketone which is readily converted into the isomeric carvacrol.† These results suggest that limonene contains a six-carbon ring to which *para*methyl and isopropyl groups are linked. Inactive limonene has now been obtained synthetically by a method which leaves no doubt as to its constitution.

The Synthesis of Limonene.—Terpineol together with terpin and limonene have been synthesized by Perkin jun.‡ with the aid of the Barbier-Grignard reagent. The starting point is δ-ketohexahydrobenzoic acid, which is obtained as follows:

$$2C_2H_5OOC \cdot CH_2 \cdot CH_2I + Na_2C\begin{matrix}CN\\COOC_2H_5\end{matrix}$$

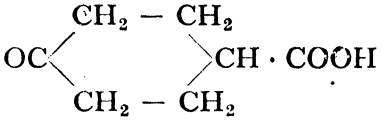
β-Iodopropionic ester

$$\rightarrow \quad \begin{matrix}C_2H_5OOC \cdot CH_2 \cdot CH_2 & CN\\ & \diagdown C \diagup \\ C_2H_5OOC \cdot CH_2 \cdot CH_2 & COOC_2H_5\end{matrix}$$

γ-Cyanopentan-αγε-tricarboxylic ester

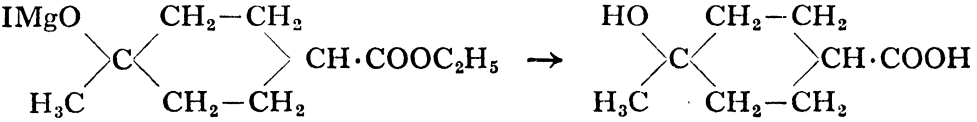
$$\xrightarrow{HCl} \quad \begin{matrix}HOOC \cdot CH_2 \cdot CH_2 & COOH\\ & \diagdown C \diagup \\ HOOC \cdot CH_2 \cdot CH_2 & COOH\end{matrix}$$

Boil with acetic anhydride and distil →



δ-Ketohexahydrobenzoic acid

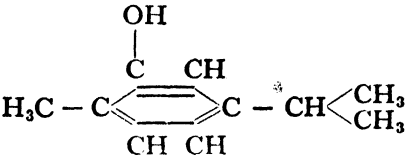
The ester of this acid reacts with magnesium methyliodide, the ketonic group being preferentially attacked, and on hydrolysis δ-hydroxyhexahydro-*p*-toluic acid is obtained:



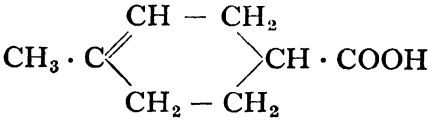
‡ *Trans.*, 1904, **85**, 138, 416; 1906, **89**, 1640; 1907, **91**, 372.

* See p. 128.

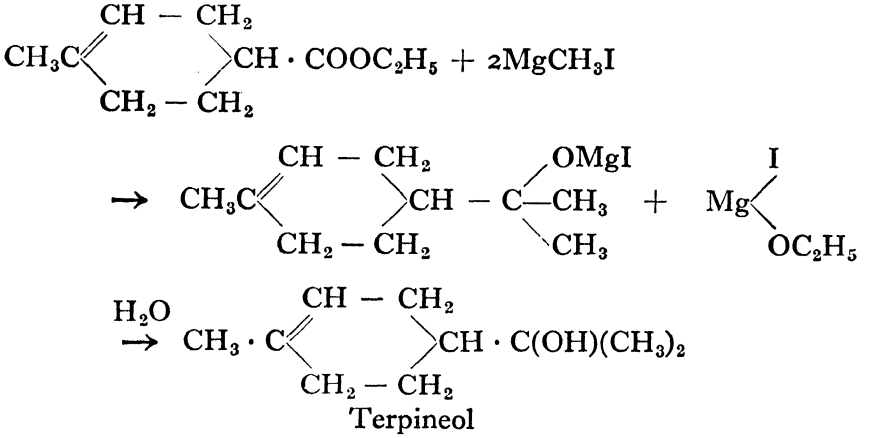
† Carvacrol has the constitution:—



By the action of fuming hydrobromic acid, δ-bromohexahydro-*p*-toluic acid is formed which, on treatment with weak alkalies, gives 3-tetrahydro-*p*-toluic acid:

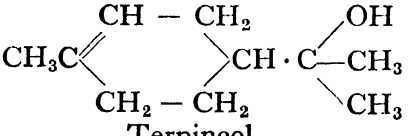


When the ester of this acid is treated with magnesium methyliodide the tertiary alcohol, terpineol, is obtained:

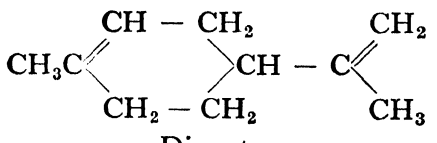


Terpineol

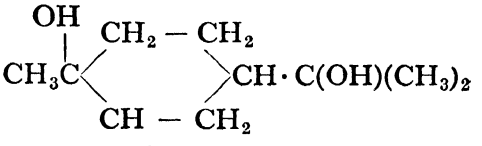
Terpineol is transformed into *dl*-limonene (dipentene) by dehydrating agents, e.g. potassium hydrogen sulphate, and into terpin hydrate by shaking with dilute sulphuric acid, or directly from the original cyclohexanone ester by the action of excess of magnesium methyliodide:



Terpineol



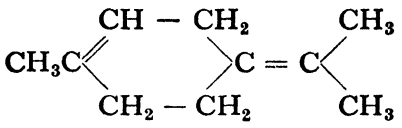
Dipentene



Terpin

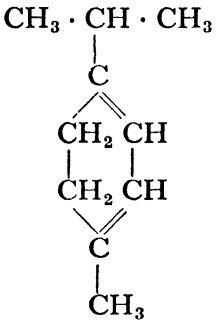
The alternative method by which water may be eliminated from terpineol, namely between the groups attached to carbon atoms 4 and 8, would lead to the production of a terpene devoid of an asymmetric carbon atom.

Terpinolene

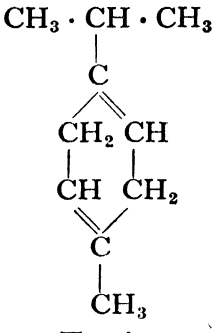


is an artificial inactive terpene first obtained by Wallach from pinene. It has not yet been observed in natural essential oils. It is produced when terpin hydrate, terpineol, or cineol are boiled with dilute sulphuric acid, and by heating pinene with concentrated sulphuric acid. With bromine, terpinolene forms a dibromide, $C_{10}H_{16}Br_2$, and a tetrabromide, $C_{10}H_{16}Br_4$, from which it can be regenerated in purity by reduction with zinc dust and alcohol.* Terpinolene is an unstable terpene, and is readily converted into terpinene by the action of dilute acids.

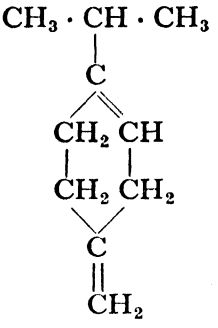
The Terpinene Group.—This group embraces the following three terpenes:



α-Terpinene



γ-Terpinene



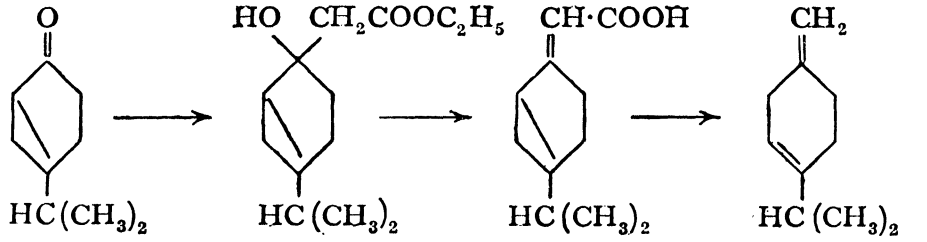
β-Terpinene

Of these terpinenes the α and γ compounds have been found in essential oils, while the β compound has hitherto only been obtained synthetically. Natural terpinene and the terpinene artificially prepared from other terpenes or terpene alcohols represent a mixture of the α and γ terpinenes, and the isolation of a pure α or γ form has not yet been accomplished. Natural terpinene occurs in cardamom oil, coriander oil, and ajowan oil. It is formed when dipentene, terpene, phellandrene, or cineol are boiled with dilute sulphuric acid and also when pinene is shaken with a little concentrated sulphuric acid.

β-terpinene was obtained synthetically by Wallach † from sabinaketone (p. 126) by condensation with bromacetic ester in the presence of zinc (Reformatsky's reaction). After hydrolysis, the

* *Ber.*, 1909, **42**, 4644. † *Ann.*, 1907, **387**, 68.

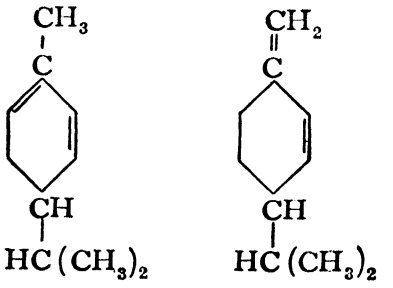
product is converted into an unsaturated acid by heating with acetic anhydride. On heating this unsaturated acid alone, it loses carbon dioxide and water and gives β-terpinene:



Sabinaketone β-Terpinene

This method has been frequently used for the replacement of the oxygen of a cyclic ketonic group by the unsaturated side chain (: CH_2).

The Phellandrene Group. — In 1904 Wallach * published the results which he had obtained in his exhaustive investigation of phellandrene—obtained from water-fennel oil (*Phellandrium aquaticum*)—and pointed out that two isomeric phellandrenes are present in this oil.



Phellandrene β-Phellandrene
(2 : 5-Dihydrocymene)[2]

The structure of the β form was later confirmed by Wallach † by its synthesis from Δ : 2-isopropylcyclohexanone after the manner of that of β-terpinene.

Sylvestrene and Carvestrene.—Sylvestrene has been found in Indian, Swedish, and Russian turpentine oil, and oil of pine needles. It is dextrorotatory, and possesses a pleasant odour resembling that of lemons.

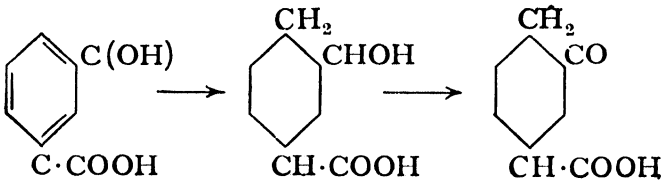
* *Ann.*, 1904, **336**, 9. † *Ann.*, 1905, **340**, 1; **343**, 29.

Carvestrene is an artificial compound which was first obtained by Baeyer * by the distillation of vestrylamine hydrochloride.

$$C_{10}H_{17}NH_2 \cdot HCl = C_{10}H_{16} + NH_4Cl$$

Both sylvestrene and carvestrene give a deep blue colour when dissolved in acetic anhydride and treated with concentrated sulphuric acid. This property is not shown by any other terpene, and since sylvestrene and carvestrene are the only menthadienes of the *meta* series it is very probable that carvestrene is the inactive form of sylvestrene.

Carvestrene has been synthesized by Perkin junior and Tattersall,† by the action of magnesium methyliodide on the ester of cyclohexanone-3-carboxylic acid in a similar manner to that of dipentene. Later ‡ the intermediate tetrahydro-*m*-toluic acid was resolved and the dextrorotatory terpene prepared from it was found to be identical in every respect with *d*-sylvestrene. Cyclohexanone-3-carboxylic acid was prepared by oxidizing the hexahydro derivative obtained by the reduction of *m*-hydroxybenzoic acid:

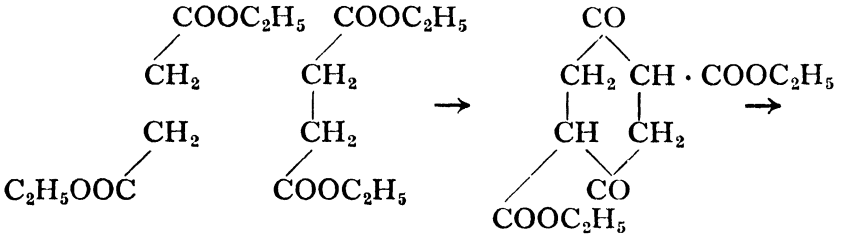


Synthetic Monocyclic Terpenes.—The first complete synthesis of a monocyclic terpene was carried out by Baeyer in 1893, and although more convenient methods have been devised in recent years, yet on account of the richness of the field explored, his synthesis must be regarded as classical. In the presence of metallic sodium, two molecules of diethylsuccinate (i) condense to form succino-succinic ester (ii) — a diketohexamethylene dicarboxylic ester. When the latter is treated with sodium ethoxide and isopropyl iodide a monoisopropyl derivative (iii) is formed which, on repetition of the process but using methyliodide in place of isopropyliodide, gives a methyl-isopropyl derivative (iv). On treatment with concentrated sulphuric acid methyl-isopropyl diketocyclohexane (v) is obtained, which may be reduced to the alcohol (vi). On bromination with strong hydrobromic acid a dibromo derivative (vii) is

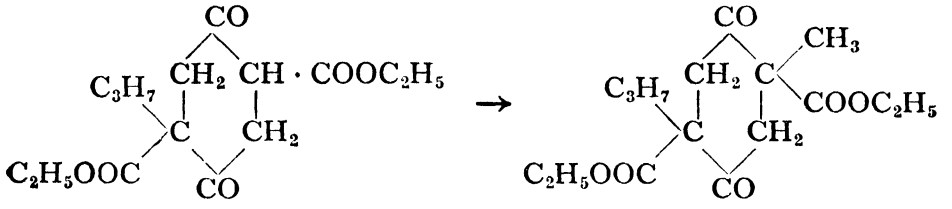
* *Ber.*, 1894, **27**, 1915, 3485; 1896, **29**, 2796.

† *Trans.*, 1907, **91**, 480. ‡ *Proc. Chem. Soc.*, 1910, **26**, 97.

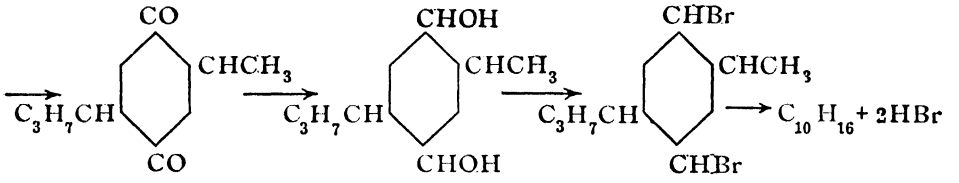
formed, from which a menthadiene of uncertain constitution is obtained on removing two molecules of hydrogen bromide by means of quinoline.



(i) (ii)



(iii) (iv)



(v) (vi) (vii)

The syntheses of limonene and carvestrene by Perkin junior have already been dealt with. These methods have been extended in several directions which may be briefly summarized as follows.*

1. From the toluic acid by reduction to the hexahydro derivative and bromination. On removal of hydrogen bromide an $\alpha\beta$-tetrahydrotoluic acid is obtained. The ester of the latter is treated with magnesium methyliodide, and the resulting menthadiene contains a conjugated double bond. By a similar process, but using the saturated hexahydrotoluic acids, the corresponding menthanols and menthenes have been obtained.

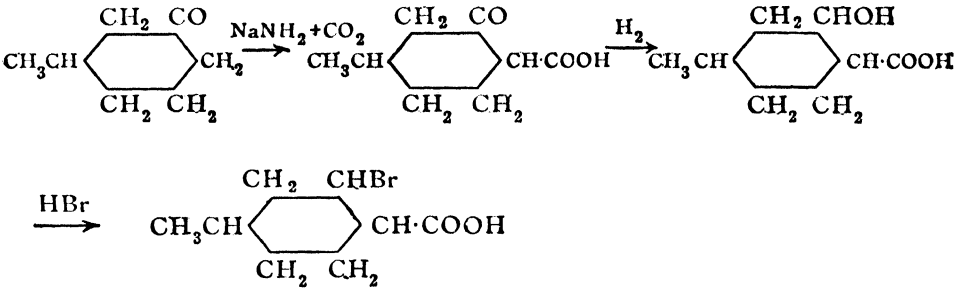
2. From the hydroxytoluic acid, which is reduced to the hexahydro derivative and thence into the bromohexahydro acid. The latter is then treated as above.

3. From the hydroxybenzoic acid by complete reduction and

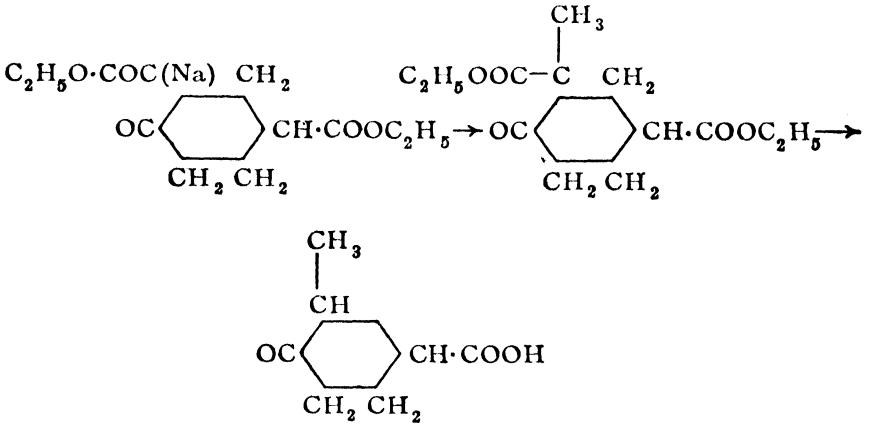
* "Synthesis of the Terpenes", by W. H. Perkin, *The Perfumery and Essential Oil Record*, 1912, 3, 149.

oxidation to the ketonic acid. Magnesium methyliodide attacks the ketonic group, and the methylhydroxy acid thus formed is converted into the bromo acid and thence to the unsaturated acid (c.f. carvestrene).

4. By the action of sodamide and carbon dioxide on the methylcyclohexanone. The resulting ketonic acid is then reduced to the hydroxyacid and thence through the bromo acid to the unsaturated acid.*



5. From cyclohexanone-2 : 4-dicarboxylic acid, the sodium derivative of which gives methylcyclohexanone carboxylic acid on treatment with methyliodide:

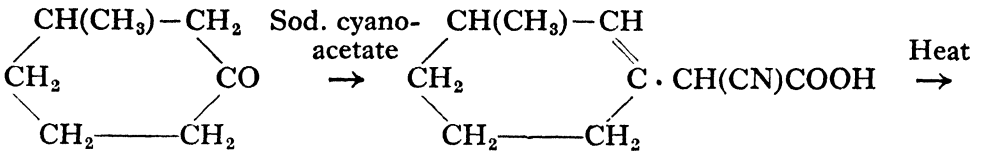


The ketonic group is reduced and the bromo acid prepared in the usual manner. Carvestrene has been synthesized in this way.†

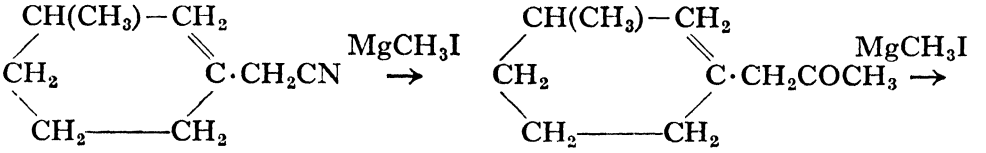
Hawarth and Fyfe ‡ have prepared menthadienes allied to the monocyclic terpenes by the application of the well-known method for the conversion of nitriles into ketones by the aid of the Barbier-Grignard reagent.

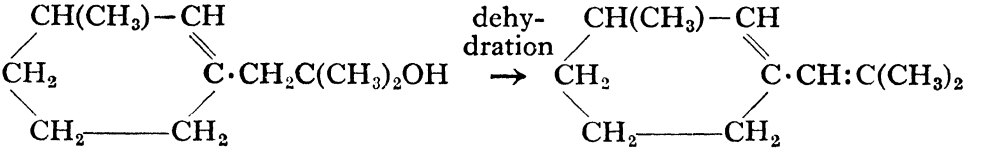
* *Trans.*, 1910, **97**, 1756; 1911, **99**, 526.

† *Trans.*, 1908, **29**, 1876. ‡ *Trans.*, 1914, **105**, 1659.

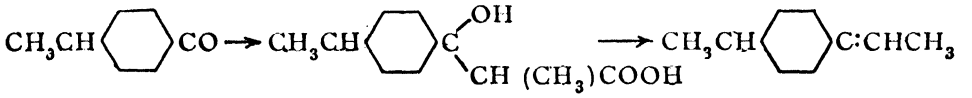


1 : Methylcyclohexan 3-one

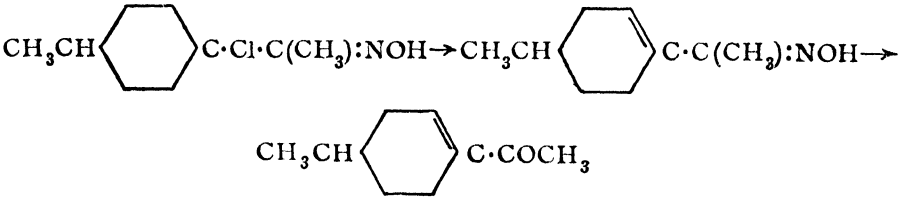




The synthesis of β-terpinene already referred to (p. 118) illustrates another synthetic method which has been elaborated by Perkin junior and Wallach.* Cycloketones are combined with α-bromopropionic ester in the presence of zinc (Reformatsky's reaction). The free acid on heating loses carbon dioxide and water, giving an unsaturated compound, e.g.



The nitrosochloride of the latter gives the oxime on elimination of hydrogen chloride and on hydrolysis yields the ketone.



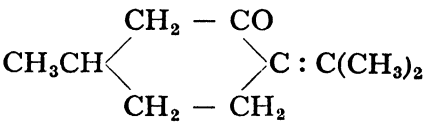
The menthanol and menthadiene are obtained on treating the latter with magnesium methyliodide.

The Menthenes and their Derivatives.—The monocyclic terpenes so far considered contain two double bonds, and are consequently termed menthadienes. A number of monocyclic terpenes of the general formula $C_{10}H_{18}$ are known which contain one double bond and are consequently termed menthenes. The

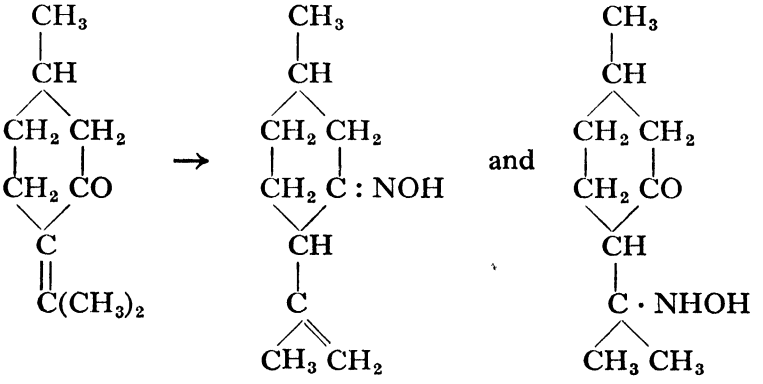
* *Trans.*, 1910, **97**, 1429; 1911, **99**, 118; *Ann.*, 1910, **374**, 198; 1911, **379**, 131.

parent hydrocarbons are not very important, but a few of their derivatives merit consideration.

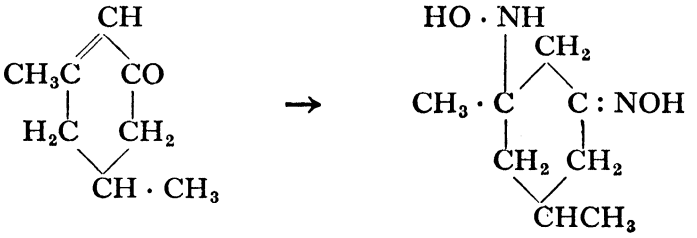
Pulegone, Δ-4 : (8)-menthene-3-ketone



is the chief constituent of oil of pennyroyal (*Mentha pulegium*). It was isolated by Beckmann and Pleissner in 1891, investigated by Semmler, and ultimately synthesized by Tiemann and Schmidt in 1896.* With hydroxylamine it forms both an oxime and an oxamino ketone, and Wallach has shown that this reaction is characteristic of cyclic ketones when the double bond is in the side chain. When the double bond is in the nucleus the so-called oxamino oximes are formed, in which one hydroxylamine molecule forms an additive compound at the double bond, and the other attaches itself to the ketone group, e.g.



Pulegone — Isopulegone oxime † — and — Oxamino pulegone



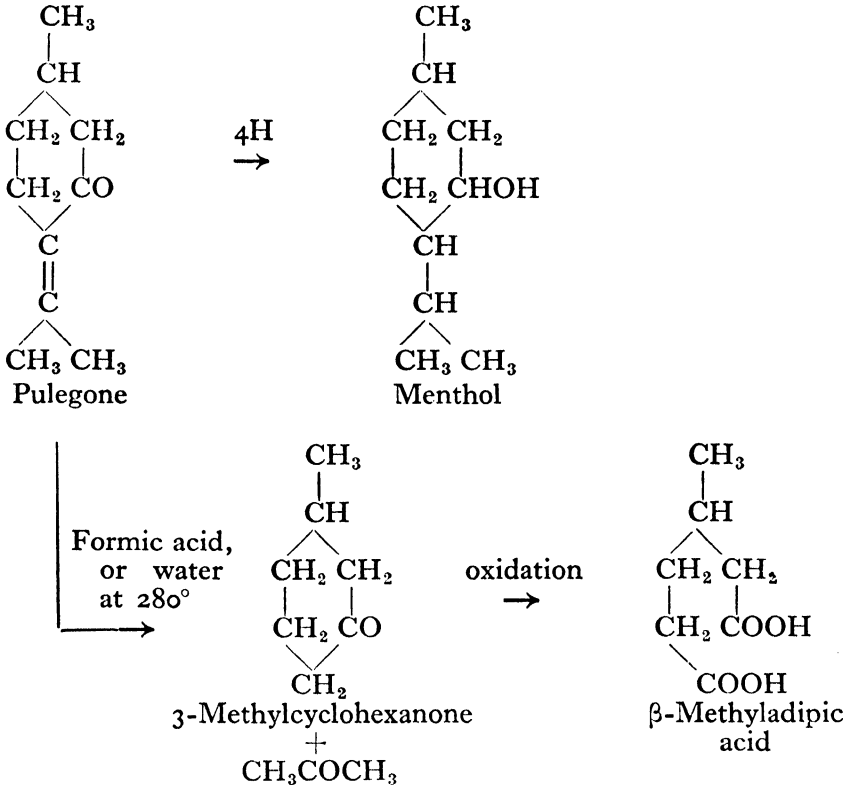
An oxamino oxime

On reduction pulegone is converted into menthol, and on oxida-

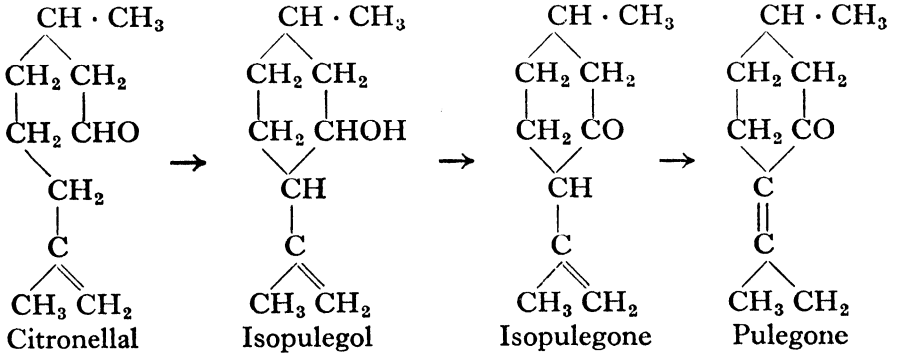
* *Ber.*, 1896, **29**, 913; 1897, **30**, 22.

† By the action of hydroxylamine the double bond moves with the formation of the oxime of isopulegone.

tion into acetone and 3-methylcyclohexanone, further oxidation of the latter giving β-methyladipic acid:



When citronellal is heated with acetic anhydride it gives isopulegol. On oxidation of the latter isopulegone is obtained, which on treatment with baryta undergoes isomeric change to pulegone: *

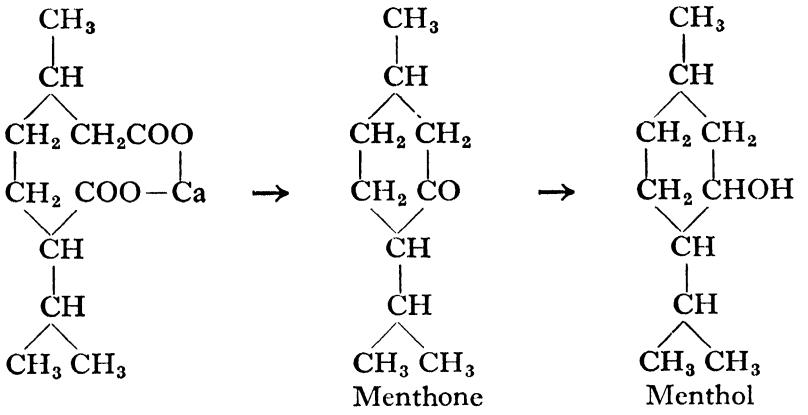


Menthone and Menthol.—Menthone occurs in Japanese, American, and Russian peppermint oils. It is known in two optically active modifications, of which the lævo form may be obtained

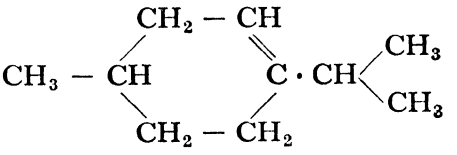
* Tiemann and Schmidt, *Ber.*, 1897, **30**, 32.

by the oxidation of menthol with potassium dichromate and sulphuric acid below 50°. Concentrated sulphuric acid converts lævomenthone into dextromenthone in the cold.

Synthetic menthone was obtained by Kötz and Schwarz * by distillation of the calcium salt of β-methyl-α′-isopropylpimelic acid:



Lævomenthol is the principal constituent of peppermint oil. A dextromenthol has been obtained by reducing the menthone from bucco oil. Several synthetic menthenols (menthols) have been synthesized by Perkin junior from the hexahydrotoluic acids as already indicated (p. 120). On dehydration of lævomenthol a dextromenthene of the constitution shown below has been obtained, and this structure has been verified by its synthesis from 1:4-methylcyclohexanone by Wallach.†



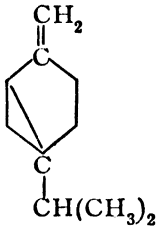
C. THE DICYCLIC TERPENES

1. **The Sabinane or Tanacetane Group.**—The closely related compounds of this group, the most important representative of which is thujone or tanacetane, contain both a trimethylene and a pentamethylene ring, and can be converted into trimethylene carboxylic acids by oxidation. Sabinene and the two thujenes must contain the same carbon skeleton, and differ

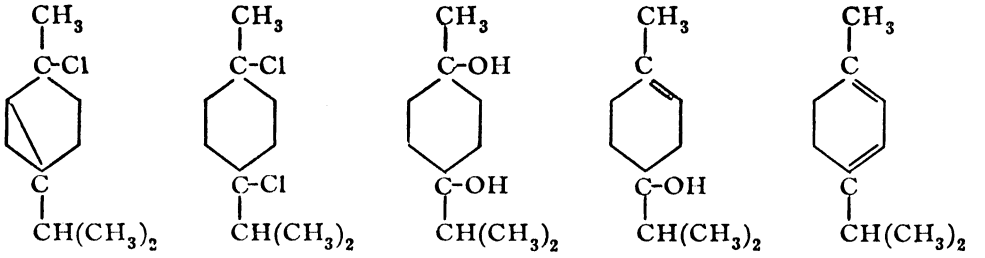
* *Ann.*, 1907, **357**, 206. † *Ber.*, 1906, **35**, 2504.

only by the position of the double linkage since they all yield the same saturated dicyclic hydrocarbon — $C_{10}H_{18}$, sabinane or thujane—on gentle reduction.*

Sabinene.—The dextro form of this compound has been found in Ceylon cardamom oil and majoram oil. With dry hydrochloric acid it yields sabinene hydrochloride, but with moist acid it gives terpinene dihydrochloride. With cold dilute sulphuric acid it gives terpinenol and 1 : 4-terpin, while on heating it gives terpinene: †

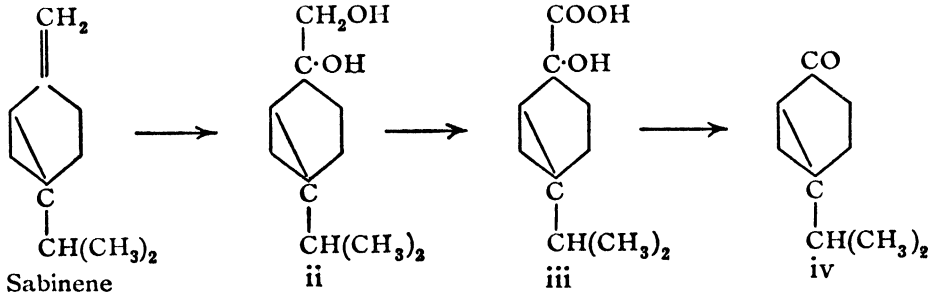


Sabinene



Sabinene hydrochloride | Terpinene dihydrochloride | 1 : 4-Terpin | Terpinenol | α-Terpinene

On oxidation with potassium permanganate sabinene glycol (ii) is first formed, which is then oxidized to sabineric acid (iii) and further to sabina ketone (iv).

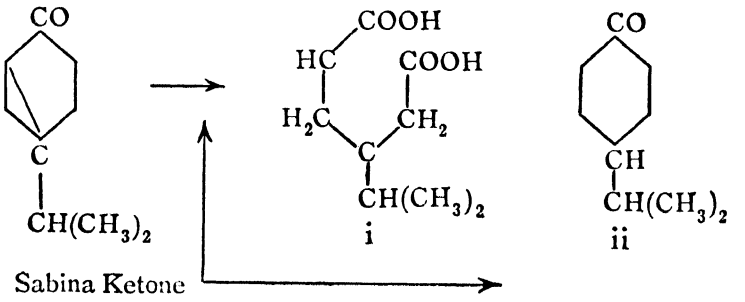


Sabinene | ii | iii | iv

On treating this cyclic ketone with warm aqueous or alcoholic sulphuric acid the trimethylene ring is broken and 2-isopropylcyclo-

* Tschugaeff and Formin, *C. r.*, 1910, **151**, 1058.
† Wallach, *Ann.*, 1907, **350**, 165.

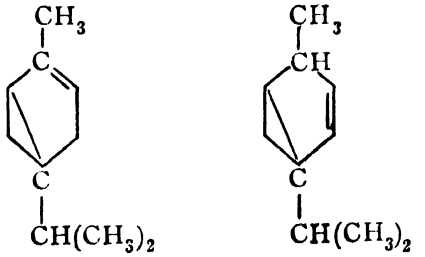
hexanone (ii) is obtained. Further disintegration leads to the formation of α-tenacetone-dicarboxylic acid (i).



The Thujenes.—These terpenes are obtained from the ketone thujone, which, according to Wallach, exists in isomeric forms. Thujone is found in thuja, wormwood, and sage oils and is lævo-rotatory. The dextro form is found in tansy oil and some wormwood oils. The oxime of thujone gives thujylamine, $C_{10}H_{17}NH_2$, on reduction, and the hydrochloride of this base yields thujene on distillation. An alternative method is due to Tschugaeff,* according to which thujone is reduced to thujyl alcohol and converted into its xanthicmethyl ester by treating the sodium compound of the alcohol with methyliodide and carbon disulphide. On dry distillation a thujene (β) is obtained which is apparently not identical with that obtained by the first method.

$$C_{10}H_{17}O \cdot CS \cdot SCH_3 = C_{10}H_{16} + COS + CH_3SH$$

Thujyl xanthic ester

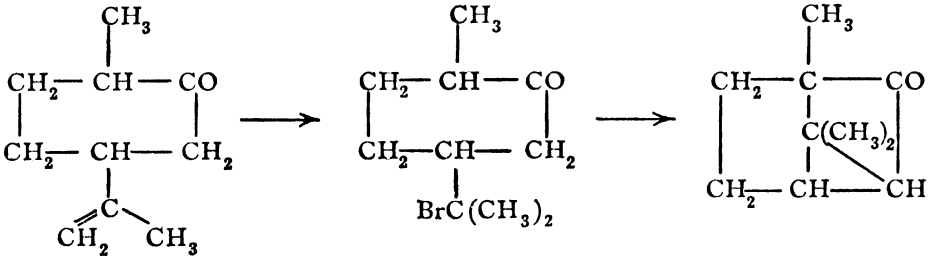


Thujene combines with hydrogen chloride to give terpinene dihydrochloride, and is converted into terpineol on treatment with dilute sulphuric acid.

* *J. Russ. Phys. Chem. Soc.*, 1904, **36**, 988.

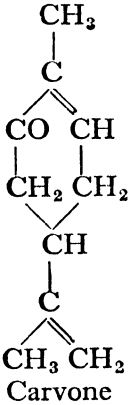
2. THE CARANE GROUP

The only member of this group which we need consider is carone. This terpene does not occur in nature, but has been obtained by the action of methylalcoholic potash on dihydrocarvone hydrobromide:



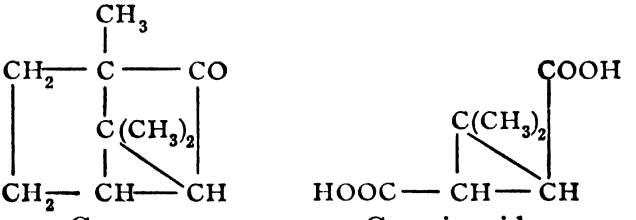
Dihydrocarvone — Carone

Dihydrocarvone is obtained by the action of mild reducing agents, such as zinc dust and alcohol, on carvone. Carvone is found as the *dextro* form in dill and carraway oil, and as the *lævo* form in spearmint and kuromoji oil, and has the constitution:



Carvone

Carone has an odour somewhat resembling camphor and peppermint. It gives caronic acid on oxidation, and the identity of this acid with dimethylcyclopropane-dicarboxylic acid has been established by its synthesis by Perkin junior and Thorpe.*



Carone — Caronic acid

* *Trans.*, 1899, **75**, 48.

THE PINANE GROUP

The Pinenes.—Pinene is a very common constituent of essential oils, and is the chief ingredient of the turpentine oils. Turpentine, the resinous juice exuding from various coniferæ, consists of a solution of resins in turpentine oil. The oil is volatile in steam while the resin (colophany) remains behind. The American, Algerian, and Greek turpentine oils contain chiefly *dextro*pinene, while the French and Spanish oils contain the *lævo* form. In most cases pinene is accompanied by small quantities of a closely related terpene of higher boiling-point. This is especially the case with the turpentine oils, and this related terpene is termed β-pinene to distinguish it from the ordinary or α-pinene.

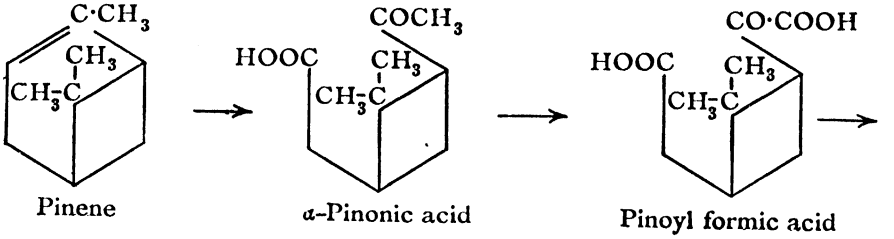
(*dl*)-α-**Pinene.**—The natural oil almost invariably contains traces of β-pinene, which may be removed by making use of the fact that α-pinene alone gives a nitrosochloride with nitrosyl chloride. The nitrosochloride is then decomposed by aniline, or by boiling it with sodium acetate and glacial acetic acid.

Pinene combines with two atoms of chlorine or bromine, and therefore the pinene molecule contains one double bond. By the action of moist hydrogen halides, pinene is converted into dipentene dihydrohalides, while monohalogen hydrates are obtained with perfectly dry acid. These, however, like the halogen additive products, no longer contain the pinene ring, the hydrogen haloids having given rise to borneol derivatives. This easy transition of pinene into borneol and isoborneol has been utilized industrially for the production of synthetic camphor from oil of turpentine.

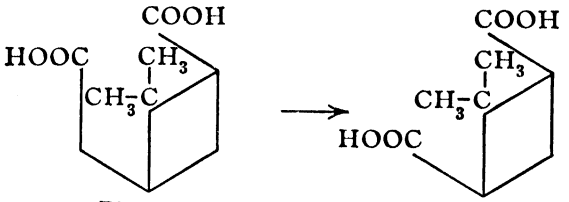
The oxidation products of pinene have been examined in detail by Baeyer, Tiemann, and Wagner, and it is to these investigators that we owe our knowledge of the structure of the pinene molecule. By the action of moist oxygen Sobrero obtained pinol hydrate or sobrerol, $C_{10}H_{16}(OH)_2$. By means of permanganate solution Baeyer * obtained α-pinonic acid, $C_{10}H_{16}O_3$, and pinoylformic acid, $C_{10}H_{14}O_5$, both of which are ketonic acids. As both acids yield the same pinic acid, $C_7H_{12}(COOH)_2$, on further oxidation, Baeyer concluded that they contain respectively a methyl ketone, $COCH_3$, and an α-ketonic acid, $CO \cdot COOH$, group. By the oxidation of α-hydroxy-pinic acid, obtained through the α-bromo acid, norpinic acid—a derivative of cyclobutane—is obtained, and this is the key to the problem of the structure of pinene. Like carone which gives caronic

* *Ber.*, 1896, **29**, 1907.

acid, and therefore contains a cyclopropane nucleus, pinene must contain a bridged ring, of which one part consists of four carbon atoms.

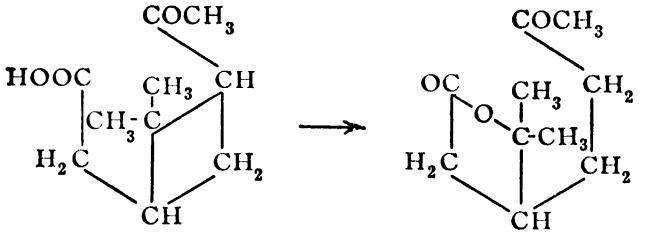


Pinene — α-Pinonic acid — Pinoyl formic acid

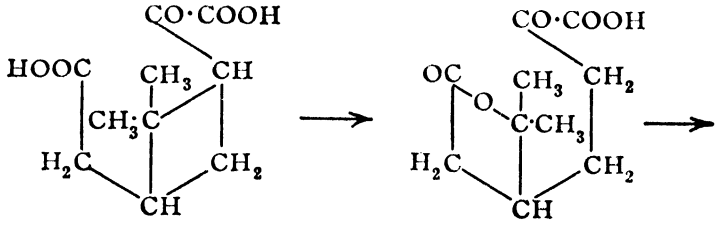


Pinic acid — Norpinic acid

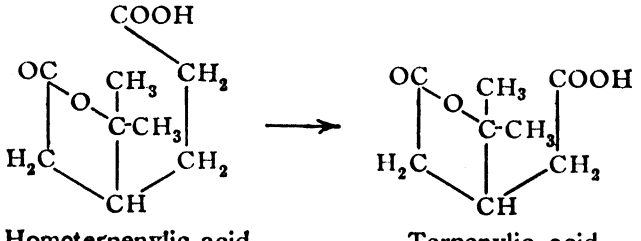
On heating with acids, α-pinonic acid and pinoyl-formic acid are transformed as follows:



α-Pinonic acid — Methyl Ketone of Homoterpenylic lactone



Pinoyl formic acid — Homoterpenyl formic acid



Homoterpenylic acid — Terpenylic acid

Simonsen * synthesized homoterpenylic, terpenylic, and terebic acids by the action of magnesium methyliodide on β-acetyl-adipic, β-acetyl-glutaric, and β-acetyl-succinic esters respectively. The reactions are so similar that only one of them need be formulated:

$C_2H_5OOC \cdot CH_2 \cdot CH(COCH_3) \cdot CH_2COOC_2H_5$

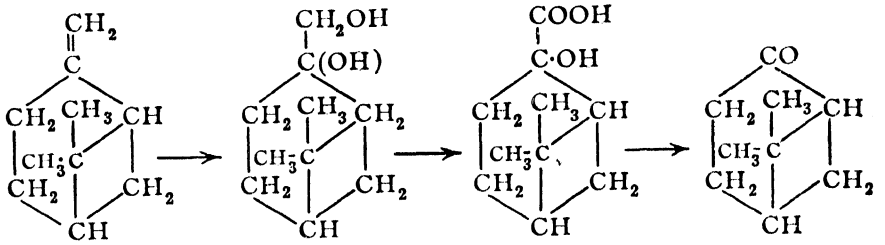
Ethyl β-acetylglutarate

$$\xrightarrow{MgCH_3I} C_2H_5OOC \cdot CH_2 \cdot CH[C(CH_3)_2 \cdot OMgI]CH_2COOC_2H_5$$

$$\rightarrow \begin{matrix} C(CH_3)_2 - CH \cdot CH_2 \cdot COOC_2H_5 \\ | \quad\quad\quad | \\ O - CO - CH_2 \end{matrix}$$

Ethyl terpenylate

β-Pinene is found together with α-pinene as already described. It has also been observed in lemon oil, coriander oil, hyssop oil, and the oil of Siberian pine needles. With hydrochloric acid it gives a mixture of bornyl chloride and dipentene dihydrochloride. On oxidation with potassium permanganate, β-pinene glycol, nopinic acid, and a ketone, nopinone, are obtained: †



β-Pinene β-Pinene glycol Nopinic acid Nopinone

Nopinone has been used for the synthesis of β-pinene, camphene, and camphor by Wallach.‡

4. THE CAMPHANE GROUP

By far the most important member of this group is the ketonic compound camphor — a substance which may exhibit manifold changes in a most interesting manner.

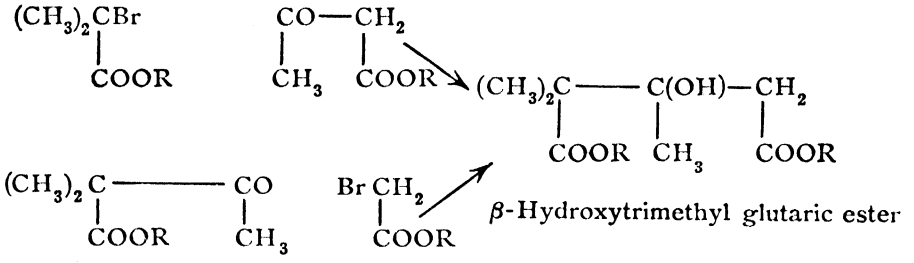
Camphor. — The study of camphor is coeval with that of organic chemistry itself, since it attracted the attention of such early

* *Trans.*, 1907, **91**, 184. † *Ann.*, 1907, **356**, 227; 1909, **368**, 9.
‡ *Ann.*, 1908, **363**, 1.

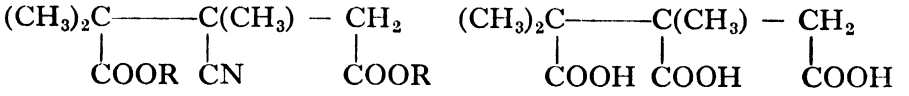
workers as Dumas and Pelouze, who derived its correct molecular formula, $C_{10}H_{16}O$. Camphor has been known from very early times, and it was introduced into Europe as a medicinal agent by the Arabians before the sixth century. As early as 1675 Lemery observed the oxidation of camphor to camphoric acid by nitric acid, and this reaction was correctly formulated by Malagati, Laurent, and Liebig.

$$C_{10}H_{16}O + 3O = C_{10}H_{16}O_4$$

The first great advance beyond this point was made by Bredt in his paper on "The Constitution of Camphoronic Acid",* and the value of his deductions was soon afterwards enhanced by the synthesis of camphoronic acid by Perkin and Thorpe in 1897.† These authors first prepared β-hydroxytrimethyl glutaric ester by the action of zinc upon a mixture of acetoacetic ester and α-bromo-isobutyric ester or upon a mixture of dimethylacetoacetic ester and monobromacetic ester:

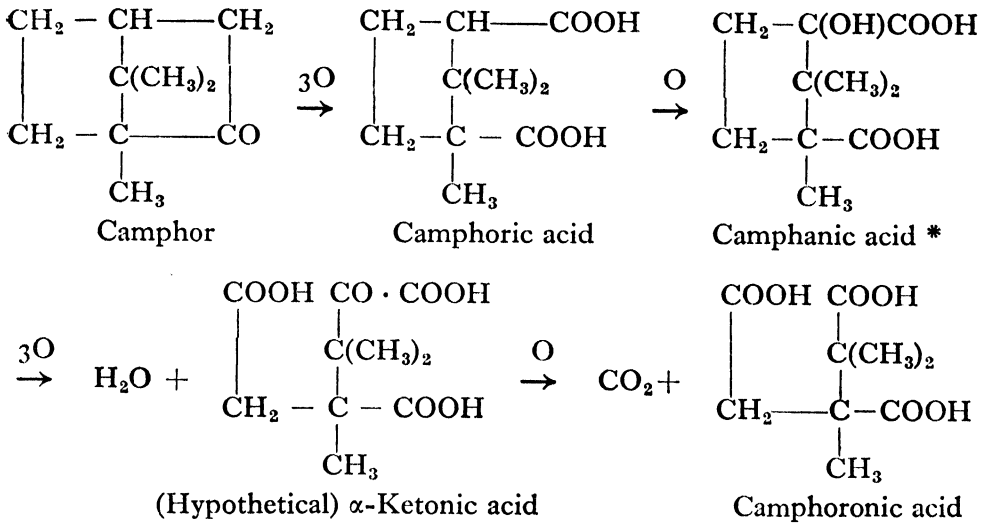


By replacing the hydroxyl group, first with chlorine and then by cyanogen, they obtained the ester of camphoronic nitrile, from which the acid itself was produced on hydrolysis:



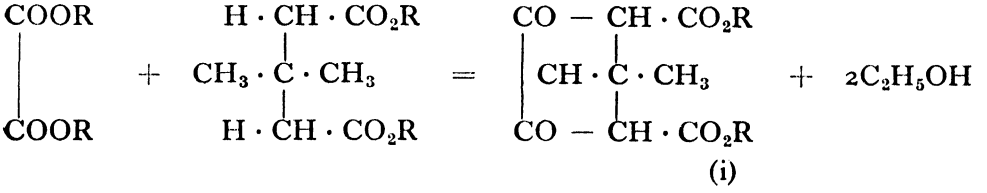
By the oxidation of camphor by nitric acid, camphoric acid, camphanic acid, and camphoronic acid may be obtained, and in the paper already mentioned Bredt suggested the following relationship among these compounds and arrived at the correct constitutional formula for camphor:

* *Ber.*, 1893, **26**, 3049. † *Trans.*, 1897, **71**, 1169.

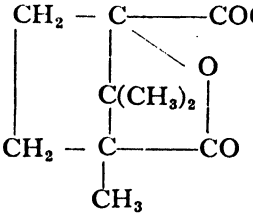


Although this formula for camphor was accepted with some reserve at the time it has now been verified by synthesis.

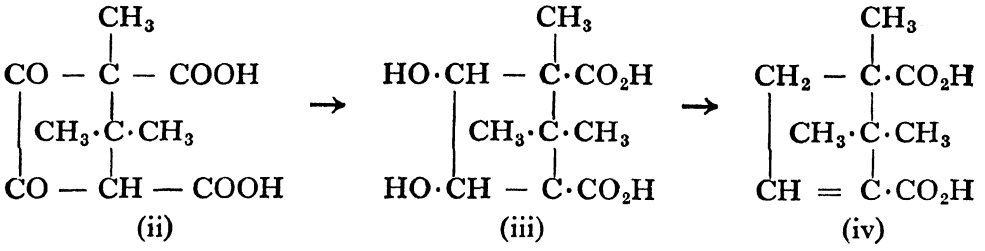
Komppa's Synthesis of Camphoric Acid.†—Ethyloxalate and ethyl ββ-dimethylglutarate were condensed by sodium ethoxide to give ethyldiketoapocamphorate (i), and a methyl group is introduced by the action of sodium and methyliodide, after which the diketo-camphoric acid (ii) was reduced to the dihydroxy acid (iii). On boiling with hydriodic acid and red phosphorus the unsaturated acid, dehydrocamphoric acid (iv) or (v), was obtained. With hydrobromic acid, β-bromocamphoric acid (vi) was formed, which on reduction with zinc dust and acetic acid gave *r*-camphoric acid (vii), which is identical with the racemic product obtained by the oxidation of camphor.



* Camphanic acid is really the lactone of this compound, viz.

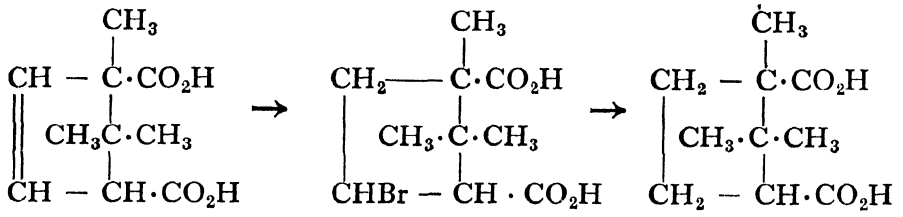


† *Ber.*, 1903, **36**, 4332; *Ann.*, 1909, **368**, 126; 1909, **370**, 209.



(ii) (iii) (iv)

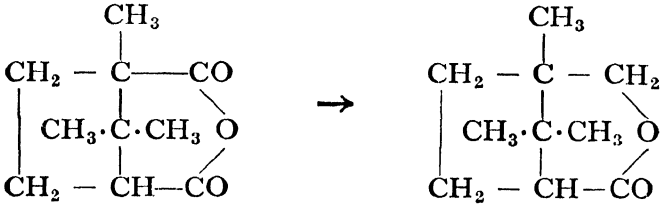
or



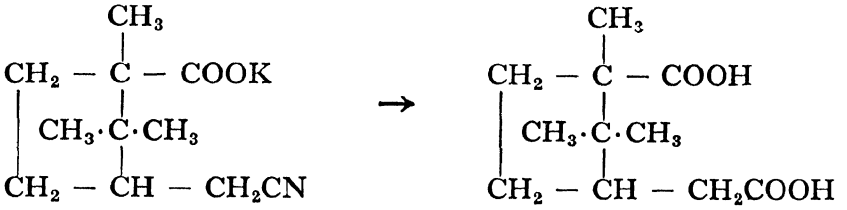
(v) (vi) (vii)

An alternate synthesis of camphoric acid was described by Perkin and Thorpe in 1906.*

The Conversion of Camphoric Acid into Camphor.—Camphoric acid was first converted into camphor by the following method. When camphoric anhydride is treated with sodium amalgam it is reduced to campholide,†



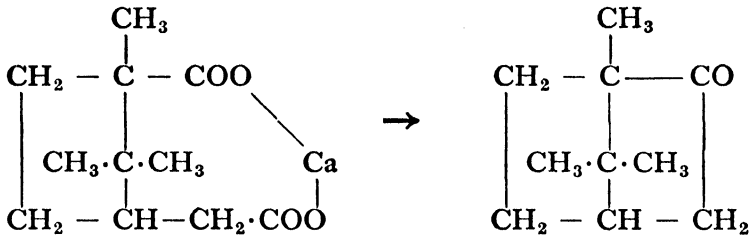
Campholide, on treatment with potassium cyanide, gives a nitrile salt which, on hydrolysis, is converted into homocamphoric acid,



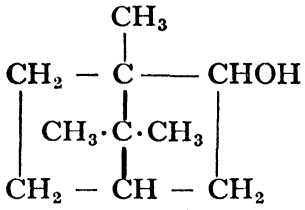
On heating the calcium or lead salt of this acid camphor is obtained:

* *Trans.*, 1906, **89**, 795.

† Haller, *Bull. Soc. Chim.*, 1896 [III], **15**, 7, 984; Forster, *Trans.*, 1896, **69**, 36.



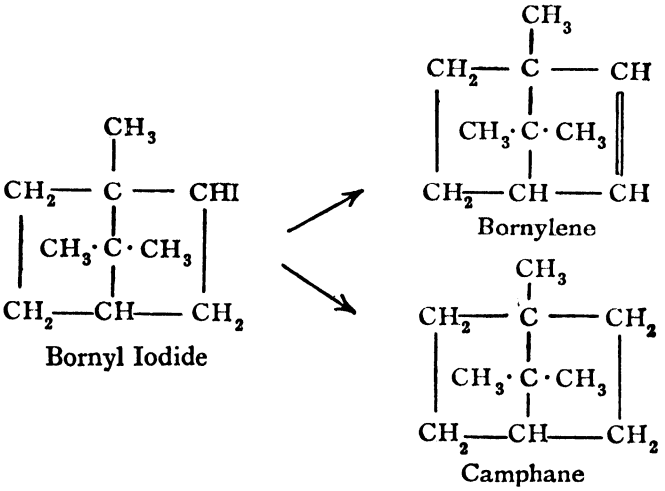
Borneol and Isoborneol.—Borneol occurs in nature in three



Borneol

modifications, *d*-borneol in *Dryobalanops camphora*, a tree growing in Borneo and Sumatra, while *l*-borneol and inactive borneol are present in the so-called baldrianic camphor. Borneol is obtained, together with traces of isoborneol, by the reduction of camphor with sodium and alcohol.* Isoborneol is probably stereoisomeric with borneol, and may be transformed into the latter by the action of sodium on a solution of borneol in benzene.

Bornylene and Camphane.—Borneol may be readily transformed into bornyl iodide, but this substance is more easily prepared by the action of hydriodic acid on pinene. When this iodide is treated with alcoholic potash at 170° bornylene † is obtained while camphane ‡ may be prepared by the reduction of bornyliodide with zinc dust and acetic acid:



Bornyl Iodide

Bornylene

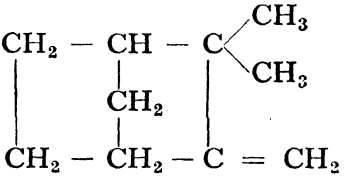
Camphane

* Wallach, *Ann.*, 1885, **230**, 225. † Wagner, *Ber.*, 1900 **33**, 2121.
‡ Aschan, *Ber.*, 1900, **33**, 1006.

Bornylene is remarkable on account of its pronounced volatility. On oxidation with potassium permanganate, camphoric acid is obtained.

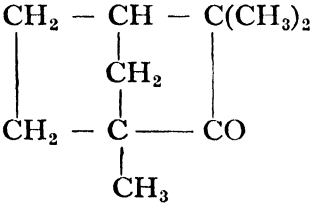
Camphene is the only natural solid terpene. It is known in dextro, lævo, and inactive modifications. The *dextro* form has been found in ginger, rosemary, and spike oils, while the *lævo* form occurs in citronella and valerian oils as well as in French and American turpentine.

The structure of camphene is not known with certainty, but the most probable formula is that of Wagner.*



Camphene

Fenchone and the Fenchenes.—Fenchone occurs naturally in two stereoisomeric forms. *Dextro*fenchone was discovered in 1890 by Wallach and Hartmann in fennel oil (*Fœniculum vulgare*), while the *lævo* form was found in 1892, by Wallach, in oil of thuja. Fenchone resembles camphor in many of its properties, but it is a liquid. It is a ketone, and on reduction gives a secondary alcohol, fenchyl alcohol, $C_{10}H_{18}O$, from which the fenchenes, $C_{10}H_{16}$, can be obtained on dehydration. The following constitutional formula for fenchone was put forward by Semmler and has recently been confirmed by the synthesis of this compound by Ruzička: †

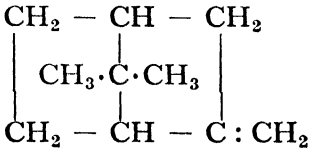


When *d*-fenchone is reduced, a lævorotatory alcohol termed *Dl*-fenchyl alcohol is obtained. With phosphorus pentachloride the latter gives *Dl*-fenchyl chloride, which is converted into *Dl*-fenchene on treatment with aniline. When these reactions are conducted without cooling *Dd*-fenchene is eventually obtained.‡

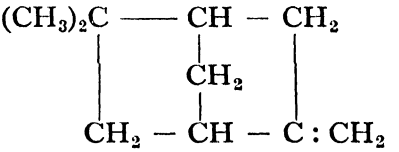
* *Ber.*, 1900, **33**, 2124; see also Semmler, *Ber.*, 1909, **42**, 246, 962.

† *Ber.*, 1917, **50**, 1362. ‡ Wallach, *Ann.*, 1898, **300**, 294; 1901, **315**, 283.

Komppa and Roschier later * proposed an alteration in the nomenclature of the fenchenes according to which Wallach's *Dl*-fenchene becomes *lα*-fenchene, and the *Dd* form becomes *Dβ*-fenchene. Racemic α-fenchene has been synthesized by these authors and the constitution (i) assigned to it. More recently Roschier † claims to have established the constitution of β-fenchene by a study of its ozonization, and of the products subsequently obtained on hydrolysis.



(i)



(ii)

THE SESQUITERPENES AND POLYTERPENES

Although a number of sesquiterpenes have been known for a considerable time, we have as yet but very little knowledge of the carbon skeleton of any of these compounds. The following tabulation embraces the more important sesquiterpenes, and the reader desiring further information should consult the treatises enumerated at the end of this chapter.

Sesquiterpene ($C_{15}H_{24}$).	Occurrence.
Cadinene.	Oil of cade, cubeb, savin, cedar wood, and camphor oil.
Caryophyllene.	Oil of cloves, copaiba balsam oil, oil of canellaalba.
Humulene.	Oil of hops.
Cedrene, and the alcohol cedrol, $C_{15}H_{25}OH$.	Oil of cedar wood.
Santalene, and the alcohol santalol, $C_{15}H_{25}OH$.	East Indian sandal-wood oil.
Zingiberene.	Oil of ginger.

In addition a number of di-, tri-, and tetra-terpenes have been isolated, but little is known of their constitution.

* *Acad. Sci. Fennicae*, 1915 [A], **7**, 1. † *Loc. cit.*, 1919 [A], **10**, 1.

The resins are closely related to the terpenes and occur with them in plants. The natural thick solutions in the essential oils are called balsams, whereas the true gum resins are amorphous, and in many cases vitreous. These products are of considerable industrial value, especially as ingredients of varnishes, but we are still almost entirely ignorant of their constitution.

NATURAL AND SYNTHETIC PERFUMES

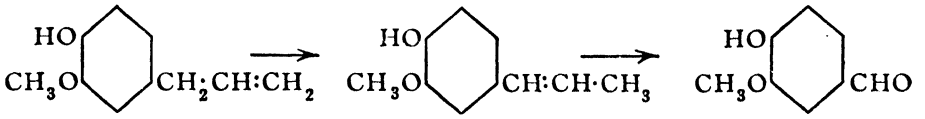
The substances which impart to many plants a particular and often characteristic odour are almost infinite in variety, and comprise some of the most interesting compounds in the domain of organic chemistry. Many of them possess a cyclic structure, while others are aliphatic compounds which may be either saturated or unsaturated; and they include representatives of such various groups as the hydrocarbons, alcohols, aldehydes, ketones, phenols, phenol ethers, acids, esters, and lactones.

Almost as soon as organic chemistry began to be seriously studied about a century ago, the esters of various acids with common alcohol were obtained. Among these compounds the odours of several flowers and fruits were speedily recognized, and it was soon discovered that the odour of pine-apple is due to ethyl butyrate, that of the pear to amyl acetate, and that of the strawberry and raspberry to mixtures of several similar esters. The odour of crushed bitter almonds was found to be due to benzaldehyde, and as early as 1847 Collas introduced nitrobenzene or "essence of mirbane" as a substitute for benzaldehyde. Even earlier than this Cahours, in 1844, found that methylsalicylate was the chief constituent of oil of wintergreen, and soon afterwards the presence of salicylic aldehyde in the flowers of the meadowsweet and cinnamic aldehyde in the barks of cinnamon and cassia was established.

One of the earliest triumphs of synthetic organic chemistry was the synthesis of coumarin—the lactone of *o*-hydroxy-cinnamic acid—by Perkin in 1868, from sodium salicylamide and acetic anhydride. Coumarin is the fragrant substance to which the perfume of the tonquin bean and woodruff are due, and it is said to be present in the artificial extract of new-mown hay.

In 1876 Tiemann and Haarmann synthesized vanillin, the sweet-smelling constituent of the vanilla pod. Vanillin is now obtained from eugenol, which is present in oil of cloves to the extent of about

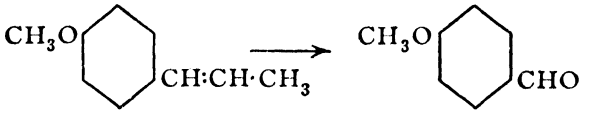
80 per cent. When eugenol is boiled with amylalcoholic potash, isoeugenol is obtained, which gives vanillin on oxidation:



Eugenol — Isoeugenol — Vanillin

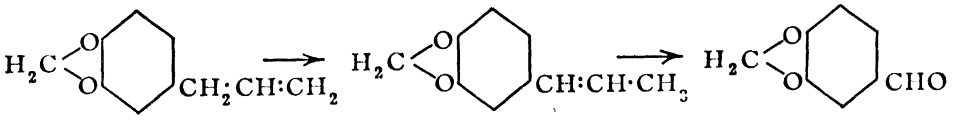
In 1888 Bauer discovered trinitro-ψ-butyltoluene, and this, as well as other strongly scented nitro compounds, has been used as artificial musk.

The flowers of may or hawthorn are believed to contain anisic aldehyde, and the latter has been prepared by the oxidation of anethole—the chief constituent of anise oil.



Anethole — Anisic Aldehyde

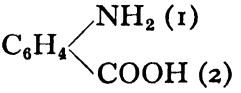
The odour of heliotrope is said to be due to piperonal, and the latter is prepared from saffrole, which is first converted into isosafrole and then oxidized:



Safrole — Isosafrole — Piperonal

Otto of roses is a mixture of which geraniol is probably the chief constituent. β-Phenylethylalcohol, obtained by the reduction of phenylacetic acid, is also frequently present. Phenylacetaldehyde possesses an intense hyacinth-like odour and is used considerably in perfumery.

The methyl ester of anthanilic acid



is present in neroli oil from orange flowers, ylang-ylang, &c., while jasmine contains indole in addition. Notwithstanding the fact that scatole (p. 182) possesses a strong fæcal odour, it is employed, as is also indole, in the preparation of synthetic perfumes.

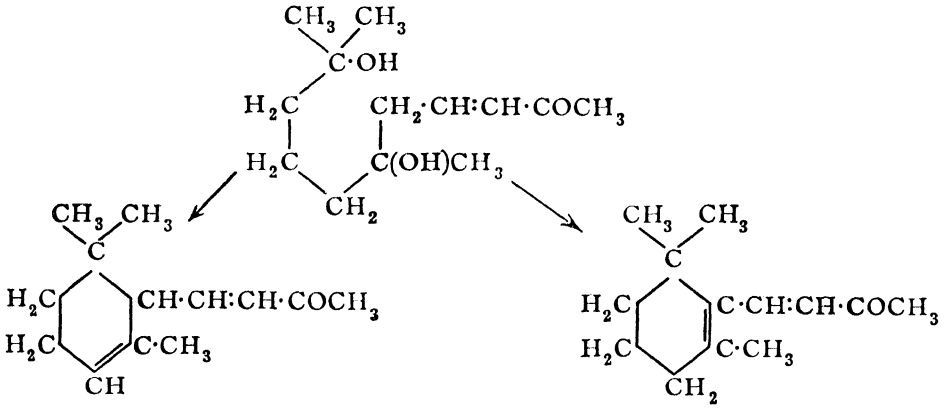
The study of the odorous principle of the violet, commenced

by Tiemann and Kruger in 1893, is one of the most interesting investigations carried out in the realm of synthetic perfumes. These chemists were unable to obtain sufficient material for their work from the flowers, but as this characteristic fragrance is possessed by the dried root of iris (orris), the latter was used as the source of the oil on which their experiments were made. To this substance, when purified, they gave the name irone, and a few years later ionone was introduced as a synthetic substitute. The latter is prepared from citral (p. 114), which undergoes the aldol condensation with acetone in the presence of baryta to give pseudoionone:

$$(CH_3)_2C{:}CH\cdot CH_2\cdot CH_2C(CH_3){:}CH\cdot CHO + CH_3COCH_3 \rightarrow (CH_3)_2C{:}CH\cdot CH_2\cdot CH_2\cdot C(CH_3){:}CH\cdot CH{:}CH\cdot COCH_3$$

Pseudoionone

On boiling with dilute sulphuric acid, α- and β-ionone are obtained:



α-Ionone β-Ionone

α-Ionone possesses the light fragrance of the violet while the odour of the β-isomer is much heavier.

Many plants belonging to the *Cruciferæ* yield volatile products which consist to a large extent of sulphur compounds, and are known by the generic name of "mustard oils". These oils do not generally pre-exist as such in the plant, but are formed by the action of a particular ferment or enzyme on a glucoside, and they consist as a rule of esters of isothiocyanic acid. A typical example is that of the oil obtained from the black mustard, *Brassica nigra* and *Brassica juncea*, and which consists almost entirely of allyl isothiocyanate, $CH_2{:}CH\cdot CH_2\cdot N{:}CS$. It is produced by the action of the ferment myrosin on the glucoside sinigrin (potassium myronate), when, besides

the volatile mustard oil, dextrose and potassium hydrogen sulphate are formed:

$$C_{10}H_{16}NS_2O_9K + H_2O = C_3H_5NCS + C_6H_{12}O_6 + KHSO_4$$

This mustard oil is also produced on a large scale synthetically by the action of allyl iodide on potassium thiocyanate in alcoholic solution, the heat employed in the operation causing the molecular transformation of the allyl thiocyanate first formed into the isothiocyanate.

Although the odorous principles of plants are frequently developed in some particular part or organ, such as the petals of the flower, they are sometimes found in both the flowers and fruit, while in other cases they are contained chiefly in the foliage. The odorous principles are generally obtained by steam distillation, but in those cases where the oil does not pre-exist in the plant, but is formed by the hydrolysis of a glucoside, as in the mustard oils, bitter almond oil, &c., a preliminary digestion with water is essential. Some of the essential oils which become impaired by heat, such as those from the orange, lemon, and bergamot, are obtained by expression. In other cases the odour of the oil is so delicate that it can only be preserved by extracting the materials with a volatile solvent or by maceration with a fixed solvent, such as an oil or fat.

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CHAPTER VII

The Amino Acids and Polypeptides

Introduction.—Excluding mineral matter and fat, the dry material of animal organisms consists of a complex assortment of nitrogenous compounds termed proteins or proteids ($\pi\rho\tilde{\omega}\tau o\varsigma$ = first, pre-eminent). The same term is also applied to somewhat similar compounds which occur in considerable quantities in all plants, especially in seeds or grain. Since the proteins furnish the material in which the vital processes of growth, repair, and reproduction are located, their study is of immense importance to physiology. These compounds are usually colloidal and non-volatile, and on this account their study offers peculiar difficulties. All the proteins contain carbon, hydrogen, nitrogen, and oxygen, while some contain phosphorus and sulphur in addition. It is doubtful if the molecular weight of any protein is known with certainty, and all that can be said is that the minimum value is probably 15,000, which is about four times as great as that of the most gigantic molecule which has yet been synthesized (p. 93). Some idea of the gigantic molecular dimensions of these compounds may be gathered from the statement that, assuming hæmoglobin contains a single atom of iron in the molecule, the minimum molecular weight is about 16,600 ($C_{158}H_{123}O_{195}N_{218}FeS_3$). It is obvious that a slight error in the analytical results makes a very great difference in the empirical formula.

If a protein, for example casein, be completely hydrolyzed by boiling with concentrated hydrochloric acid, a clear dark-coloured solution is obtained which contains a number of products, the separation of which has long taxed the chemist's ingenuity. As early as 1820 Braconnot had obtained glycine and leucine from gelatine, and in 1846 Liebig had obtained tyrosine from the decomposition products of horn. At a later date Kossel and his collaborators examined the simpler proteins, such as the protamines, but progress was very slow until Fischer, in 1901, introduced new

methods for the separation and identification of the products of protein hydrolysis.

Isolation of the Amino Acids.—The amino acids bear to the proteins a relationship recalling that of a hexose to a polysaccharose. The early attempts to explain the structure of the proteins were hampered by experimental obstacles to the separation of the complex mixture of amino acids produced on hydrolysis. With the exception of tyrosine and cystine, which are sparingly soluble in water, the major portion of the mixture remains as a syrup. Fischer made a practical advance of great importance as a result of his studies of the esters of the amino acids, substances which have the properties of aliphatic amines owing to the suppression of the carboxyl group by esterification.

For the separation of the amino acids, Fischer esterified the complex mixture, obtained on hydrolysis, with ethyl alcohol in the presence of hydrochloric acid, and after liberating the esters from their hydrochlorides by caustic soda at low temperatures or by sodium ethoxide, the esters were fractionated at 10 to 12 mm. and finally at 0·5 mm.* The esters of histidine and the diamino acids cannot be purified by distillation, while a few amino acids, e.g. tyrosine, require special methods for the liberation of their esters.

More recently Dakin † has observed that certain amino acids can be extracted from water by but slightly miscible solvents, of which butyl alcohol—now obtained as a by-product in the manufacture of acetone by the fermentation of cereals—seems to be the most useful. After hydrolysis with sulphuric acid and neutralization with baryta, the solution is concentrated in order to allow any tyrosine, which may be present, to crystallize. The solution is then extracted in a continuous apparatus at 60° to 80°. Proline and the feebly ionized monoamino acids are very easily extracted while the stronger acids and bases remain behind. By the application of this method to casein a new hydroxyamino acid, α-amino-β-hydroxyglutaric acid (β-hydroxyglutamic acid, $HOOC \cdot CH(NH_2)CH(OH)CH_2COOH$), has been obtained in quantity exceeding 10 per cent.

Foreman ‡ converts the mixture of amino acids into their dry lead salts and esterifies with absolute alcohol containing dry hydrochloric acid. The free hydrochloric acid is removed, partly by reducing the liquid to half its bulk at 40° and 15 mm., and the remainder by the addition of absolute alcohol saturated with dry

* *Ber.*, 1901, **34**, 433; *Ber.*, 1902, **35**, 2160. † *Biochem. J.*, 1918, **12**, 290.
‡ *Ibid.*, 1919, **13**, 378.

ammonia gas. After removing the alcohol in vacuo, the ester hydrochlorides are dissolved in dry chloroform and the esters liberated by shaking with anhydrous barium oxide. In this way the usual considerable loss of esters by hydrolysis is avoided.

Classification of the Amino Acids.—The following table contains the principal amino acids which have been isolated from the products of protein hydrolysis:

Monobasic Monoamino Acids

Glycine = aminoacetic acid, $(NH_2)CH_2COOH$

Alanine = α-aminopropionic acid, $CH_3\overset{+}{\underset{*}{C}}H(NH_2)COOH$

Valine = α-aminoisovaleric acid, $(CH_3)_2CH \cdot \overset{+}{C}H(NH_2)COOH$

Leucine = α-aminoisobutylacetic acid, $(CH_3)_2CH \cdot CH_2\overset{+}{C}H(NH_2)COOH$

Isoleucine = α-amino-β-methylethylpropionic acid, $(C_2H_5)(CH_3)\overset{+}{C}H \cdot \overset{+}{C}H(NH_2)COOH$

Caprine = α-aminocaproic acid, $(CH_3)(CH_2)_3\overset{+}{C}H(NH_2)COOH$

Diamino Acids

Ornithine = αδ-diaminovaleric acid, $(H_2N)CH_2 \cdot CH_2 \cdot CH_2\overset{+}{C}H(NH_2)COOH$

Lysine = αε-diaminocaproic acid, $(H_2N)CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2\overset{+}{\cdot} CH(NH_2)COOH$

Arginine = α-amino-δ-guanidovaleric acid,

$$(H_2N) \cdot \underset{\underset{NH}{\|}}{C} - NH \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot \overset{+}{C}H(NH_2)COOH$$

Dibasic Monoamino Acids

Aspartic acid = aminosuccinic acid, $HOOC \cdot CH_2 \cdot \overset{+}{C}H(NH_2)COOH$

Glutamic acid = aminoglutaric acid, $HOOC \cdot CH_2 \cdot CH_2 \cdot \overset{+}{C}H(NH_2)COOH$

Hydroxy- and Thio-amino Acids

Serine = α-amino-β-hydroxypropionic acid, $CH_2(OH)\overset{+}{C}H(NH_2)COOH$

β-Hydroxyglutamic acid, $HOOC \cdot \overset{+}{C}H(NH_2) \cdot \overset{+}{C}H(OH) \cdot CH_2 \cdot COOH$

Diaminotrihydroxydodecanic acid, $C_{11}H_{16}(OH)_3(NH_2)_2COOH$

* $\overset{+}{C}$ denotes asymmetric carbon atom.

Cysteine = α-amino-β-thiolactic acid, $CH_2(SH)\overset{+}{C}H(NH_2)COOH$

Cystine = disulphide of α-amino-β-sulphydropropionic acid,

$$\begin{array}{l} S \cdot CH_2 \cdot \overset{+}{C}H(NH_2)COOH \\ | \\ S \cdot CH_2 \cdot \overset{+}{C}H(NH_2)COOH \end{array}$$

Aromatic Amino Acids

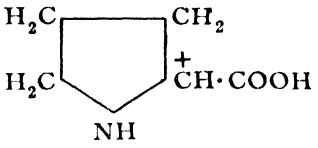
Phenylalanine = α-amino-β-phenylpropionic acid,

$$C_6H_5CH_2\overset{+}{C}H(NH_2)COOH$$

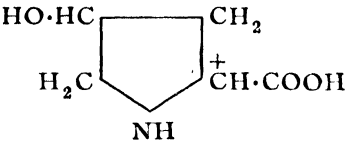
Tyrosine = α-amino-β-hydroxyphenylpropionic acid,

$$HO \cdot C_6H_4 \cdot CH_2 \cdot \overset{+}{C}H(NH_2)COOH$$

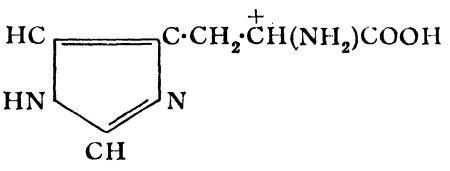
Heterocyclic Acids



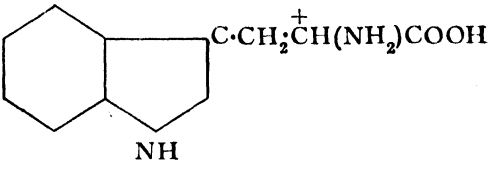
Proline = α-pyrolidine carboxylic acid



Oxyproline = β′-hydroxypyrolidine carboxylic acid



Histidine = α-amino β-iminazole-propionic acid



Tryptophane = indole α-aminopropionic acid

It will be observed that, with the exception of proline and oxyproline, all these acids contain an amino group in the α-position. In the case of proline and oxyproline the carboxyl group is adjacent to the basic NH group of the ring. In all these acids the basic amino group is more or less neutralized by the adjacent carboxyl group. Amino acids which contain an amino group in a non-adjacent position to the carboxyl group will be described in a subsequent chapter (p. 187).

The Resolution and Identification of the Amino Acids.—Nearly all the amino acids tabulated above contain one or more asymmetric carbon atoms. The early resolution of these acids had been limited by their amphoteric nature, and was easy only in the

case of aspartic acid, Piutti having shown in 1887 that asparagine is resolved by simple crystallization from water.

By suppressing the basic properties of the amino acids by benzoylating, formylating, or *p*-nitrobenzoylating the amino group, and thus encouraging their capacity to form recrystallizable salts with the natural alkaloids, strychnine and brucine, Fischer and his collaborators succeeded in resolving the *dl* forms of many of the amino acids. In this manner optically active units were obtained which became available as building materials for the construction of optically active polypeptides.

The above acyl derivatives of the amino acids, in common with other derivatives depending on the reactivity of the amino group with phenylcarbimide and benzene sulphonyl chloride, are useful for the identification as well as the isolation of their parent compounds, but the combination with β-naphthalene-sulphonyl-chloride is probably the best of all. The resulting derivatives are formed in good yield, are sparingly soluble, and crystallize well. To a minor extent, β-naphthalene-sulphonyl-chloride assumes a similar part to that played by phenylhydrazine in the sugar group.

Monobasic Monoamino Acids

A glance at the tabulation on p. 164 will show that these acids form a large part of the products of hydrolysis of most proteins. They are all crystalline, water-soluble, sweet-tasting substances which are almost insoluble in alcohol and ether. They have a neutral reaction to litmus, but form well-defined crystalline salts both with acids and bases. With the exception of glycine all these acids contain asymmetric carbon atoms, and one or other of the active forms is present in protein hydrolysis products. The acids may be synthesized by a variety of methods of which the following are the more important.

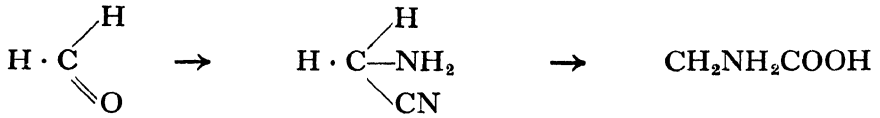
(1) By the action of ammonia on the halogen fatty acids *: e.g.

$$\underset{\text{α-brompropionic acid}}{CH_3 \cdot CHBrCOOH} \rightarrow \underset{\text{α-aminopropionic acid}}{CH_3CH(NH_2)COOH}$$

Fischer and Schmitze † have shown that good yields of halogen fatty acids are obtained by brominating the corresponding alkylmalonic acids and then converting the products into monobasic acids by distillation.

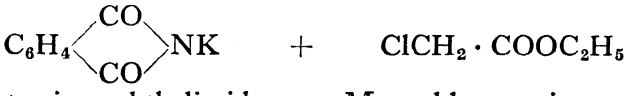
* Kolbe, *Ann.*, 1860, **113**, 220. † *Ber.*, 1906, **39**, 351.

(2) By Strecker's method, which consists in converting an aldehyde into the corresponding aminocyanhydrin, with ammonia and hydrocyanic acid, and hydrolyzing the product: e.g.

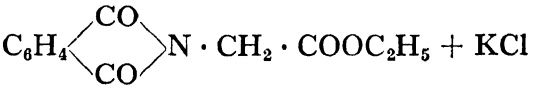


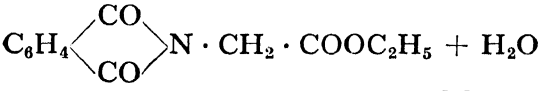
Formaldehyde Glycine*

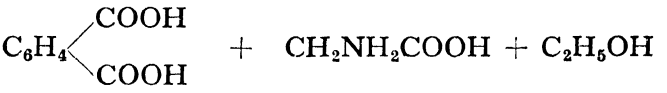
(3) By Gabriel's method.† This method consists in combining potassium phthalimide with halogen fatty acid ester and hydrolyzing the product: e.g.



Potassium phthalimide Monochloroacetic ester



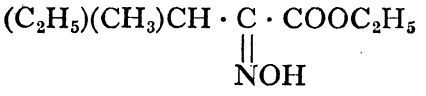




Phthalic acid Glycine

This reaction has met with very considerable application.

(4) By the reduction of the oximes or phenylhydrazones of ketonic acids with sodium amalgam or aluminium amalgam: ‡ e.g.



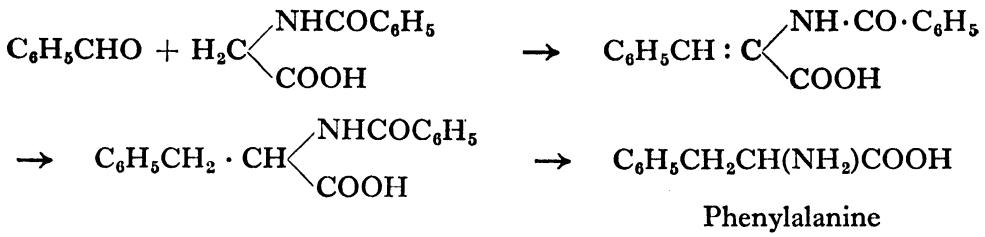
sec. Butyloximino acetic ester

Isoleucine

(5) By the method of Erlenmeyer junior.§ According to this method aldehydes or esters are condensed with hippuric acid, in the presence of acetic anhydride and sodium acetate, and the product is subsequently reduced and hydrolyzed. This recalls the well-known Perkin reaction.

* Eschweiler, *Ann.*, 1894, **278**, 237. † *Ber.*, 1889, **22**, 426.

‡ Tafel, *Ber.*, 1886, **19**, 2414; Bouveault, *Bl.*, 1904 (3), **31**, 1176; 1906 (3), **35**, 966. § *Ann.*, 1893, **275**, 1; 1899, **307**, 70, 163.



Phenylalanine

Glycine (γλυκὺς = sweet, κόλλα = glue). — Hippuric acid, the benzoyl derivative of glycine, was isolated from the urine of herbivorous animals by Rouelle as early as 1773. Glycine may be obtained in relatively large quantity by the hydrolysis of glue or gelatine, and was obtained from this source by Braconnot in 1820. Sarcosine and betaïne may be regarded as derivatives of glycine (p. 184).

Alanine.—In the synthesis of this acid by the Strecker method, a convenient modification * of the usual procedure consists in treating acetaldehyde with potassium cyanide in the presence of ammonium chloride. The acid may be resolved by fractional crystallization of the brucine salt of its N-benzoyl derivative,† or with the aid of moulds.‡ Dextroalanine is one of the principal products of the hydrolysis of fibroin—the main component of silk. Both active forms have a sweet taste.

Leucine.—The lævo form of this acid is very widely distributed in the animal kingdom, and is a substance of physiological importance. It is found in the lymphatic glands, the spleen, and especially in the pancreas. It is produced by the hydrolysis of hæmoglobin, egg-albumin, and casein, from the last of which it is usually prepared. Its solution in hydrochloric acid is dextrorotatory, but a solution of the acid in water is lævorotatory. In contact with *Penicillium glaucum*, a solution of *dl*-leucine becomes lævorotatory, owing to the destruction of the *d* modification.

Isoleucine.—The dextro form of this acid is obtained by the hydrolysis of the proteins contained in beetroot sap, cereals, potatoes, &c. A consideration of the formula of isoleucine shows that two dissimilar asymmetric carbon atoms are present in the molecule, and the following forms should therefore exist:§

Dextro-, lævo-, and *dl*-isoleucine.
Dextro-, lævo-, and *dl*-alloisoleucine.

* Delépine, *Bl.*, 1903, **29**, 1178, 1192. † Fischer, *Ber.*, 1899, **32**, 2454.
‡ M'Kenzie, Harden, *Trans.*, 1903, **83**, 428; Ehrlich, *Zentr.*, 1906, **2**, 501.
§ Ehrlich, *Ber.*, 1907, **40**, 2453.

Ehrlich * has shown that in alcoholic fermentation leucine and isoleucine give rise respectively to isoamyl alcohol and secondary butyl carbinol, which form the bulk of the fusel oil fraction. The ammonia formed in the reaction is assimilated and removed.

$$(CH_3)_2CH \cdot CH_2 \cdot CH(NH_2)COOH + H_2O$$
Leucine
$$= (CH_3)_2CH \cdot CH_2 \cdot CH_2OH + NH_3 + CO_2$$
Isoamylalcohol

$$(CH_3)(C_2H_5)CH \cdot CH(NH_2)COOH + H_2O$$
Isoleucine
$$= (CH_3)(C_2H_5)CH \cdot CH_2OH + NH_3 + CO_2$$
Secondary butylcarbinol

Diamino Acids

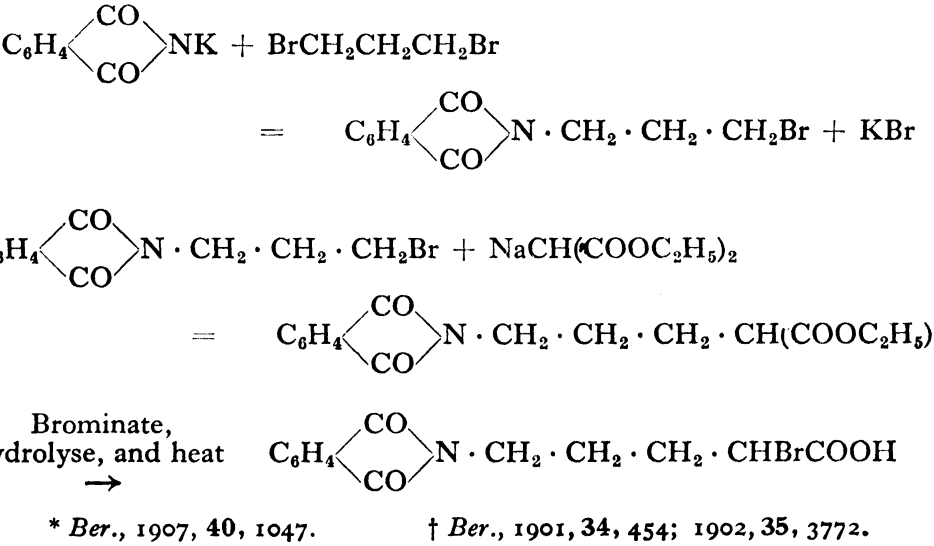
The diamino acids are strongly basic substances. They are almost invariably obtained among the hydrolytic products of the proteins.

Ornithine.—Ornithine was obtained in 1877 by Jaffe, by the hydrolysis of ornithuric acid, obtained from the excrement of birds fed on benzoic acid. Since on hydrolysis ornithuric acid yields two molecular proportions of benzoic acid to one of ornithine, its constitution is represented as dibenzoyl ornithine:

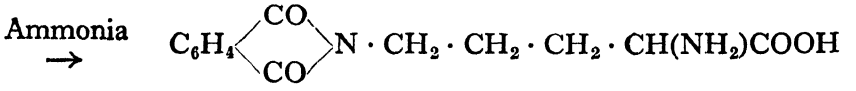
$$(C_6H_5CONH)_2 \cdot C_4H_7COOH$$

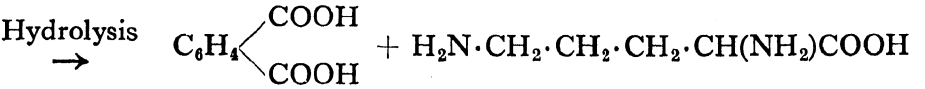
Ornithine has been synthesized by several methods, of which the following are the most important.

(1) By Fischer,† using a combination of the phthalimide and malonic ester condensations:



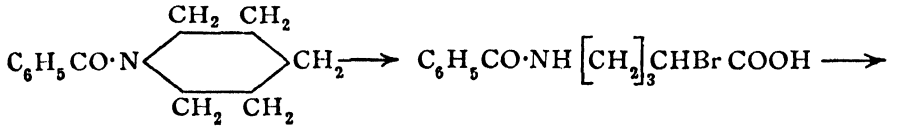
* *Ber.*, 1907, **40**, 1047. † *Ber.*, 1901, **34**, 454; 1902, **35**, 3772.

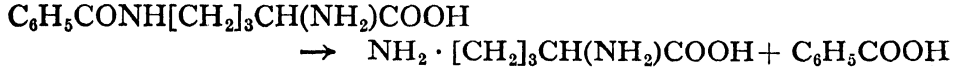




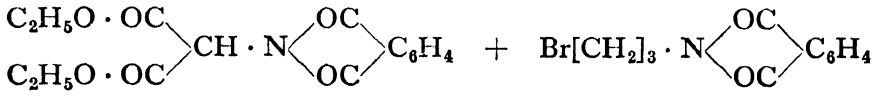
(*dl*)-αδ-diaminovaleric acid (ornithine)

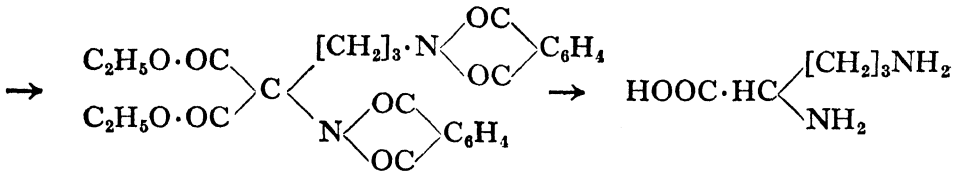
(2) When benzoyl piperidine is oxidized with potassium permanganate the ring is opened and benzoyl δ-amino valeric acid is obtained. By the action of bromine and phosphorus on the latter a bromo derivative is obtained, which on treatment with ammonia gives monobenzoyl ornithine. The latter on hydrolysis is converted into ornithine.*





(3) Sorensen's method † is somewhat similar to that first employed by Fischer. Bromomalonic ester is condensed with potassium phthalimide to give phthaliminomalonic ester. The sodium compound of the latter is then condensed with γ-bromopropylphthalimide to give phthalimino-γ-phthaliminopropylmalonic ester, which on hydrolysis gives ornithine:





Lysine.—In 1889 Drechsel obtained lysine and a substance which he termed " lysatinine " by the hydrolysis of casein with hydrochloric acid. The latter was subsequently shown to be a mixture of lysine and arginine. Ornithine, lysine, and arginine may

* Fischer and Zemplin, *Ber.*, 1909, **42**, 1022. † *Zentr.*, 1903, **2**, 34.

be precipitated from acid solution by phosphotungstic acid. This reagent also serves as a precipitant for the heterocyclic amino acids, but it does not precipitate the other amino acids derived from protein.

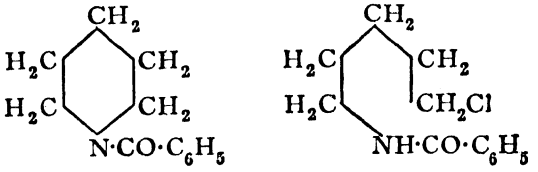
Fischer and Weigert * synthesized this acid from γ-chlorbutyronitrile and malonic ester as follows:

$$CN \cdot CH_2 \cdot CH_2 \cdot CH_2Cl + NaCH(COOC_2H_5)_2 \rightarrow CN \cdot [CH_2]_3CH(COOC_2H_5)_2$$

$$\begin{array}{l} CH_2 \cdot CH(COOC_2H_5)_2 \\ | \\ CH_2 \cdot CH_2 \cdot CN \end{array} \xrightarrow{C_2H_5ONO} \begin{array}{l} CH_2 \cdot C(NOH)COOC_2H_5 \\ | \\ CH_2 \cdot CH_2 \cdot CN \end{array}$$

$$\xrightarrow{\text{Reduction}} \begin{array}{l} CH_2 \cdot CH(NH_2)COOH \\ | \\ CH_2 \cdot CH_2 \cdot CH_2NH_2 \end{array}$$

Lysine has also been synthesized by von Braun.† For this purpose benzoylpiperidine is treated with phosphorus pentachloride, and gives the N-benzoyl derivative of the halogenated amine:



The latter is transformed into the nitrile and the acid, and the succeeding steps are analogous to those employed by Fischer and Zemplen for the synthesis of ornithine:

$$C_6H_5CO \cdot NH[CH_2]_5CN \rightarrow C_6H_5CO \cdot NH[CH_2]_5COOH$$

Arginine. — This amino acid occurs among the decomposition products of a great number of proteins. It was first obtained from lupin seedlings by Schultze and Steiger in 1887. As much as 87 per cent has been found in the spermatazoa of the salmon.

It is completely resistant to acids, but on hydrolysis by alkalies or the enzyme *arginase* it yields urea and ornithine:

$$\begin{array}{l} H_2N \cdot C \cdot NH \cdot [CH_2]_3CH(NH_2)COOH + H_2O \\ \quad \| \\ \quad NH \end{array}$$

$$= H_2N \cdot CO \cdot NH_2 + H_2N \cdot [CH_2]_3CH(NH_2)COOH$$

* *Ber.*, 1902, 35, 3772. † *Ber.*, 1909, 42, 839.

Arginine was obtained synthetically by Schultze and Winterstein by the action of cyanamide on ornithine:

$$NH_2CN + H_2N[CH_2]_3CH(NH_2)COOH = H_2N \cdot \overset{\overset{NH}{\|}}{C} \cdot NH \cdot [CH_2]_3CH(NH_2)COOH$$

It has been suggested by Robinson that ornithine and lysine may play an important part in the phytochemical synthesis of some of the plant bases, and these theories will be discussed in the chapter on the alkaloids.

Dibasic Monoamino Acids

Aspartic Acid.—Both aspartic and glutamic acids are strongly acidic and form well defined metallic salts. They are still, however, sufficiently basic to combine with acids. The lævo form of aspartic acid is frequently encountered among the hydrolytic products of proteins, but this acid is most conveniently prepared by the action of hydrochloric acid on asparagine.

Inactive aspartic acid has been obtained by the action of ammonia on fumaric acid, and the *d* acid by the action of ammonia on *l*-bromosuccinic acid. Piutti † obtained aspartic acid by the action of hydroxylamine on oxalacetic ester and subsequent reduction of the resulting isonitrososuccinic acid:

$$\begin{matrix} CO \cdot COOC_2H_5 \\ | \\ CH_2 \cdot COOC_2H_5 \end{matrix} \rightarrow \begin{matrix} HON : C \cdot COOC_2H_5 \\ | \\ CH_2 \cdot COOC_2H_5 \end{matrix} \rightarrow \begin{matrix} H_2N \cdot CH \cdot COOC_2H_5 \\ | \\ CH_2 \cdot COOC_2H_5 \end{matrix}$$

Lævoasparagine ($NH_2COCH_2CH(NH_2)COOH$), the semi-amide of aspartic acid, occurs in many plants, especially asparagus and the young shoots of beans, peas, and lupins, from which it is readily extracted by water. It is noteworthy that when an aqueous solution of equal quantities of *d*- and *l*-asparagine is evaporated a racemic compound is not formed, but the *d* and *l* forms crystallize out side by side.

Glutamic Acid.—This acid is obtained to the extent of 30 per cent by the hydrolysis of the proteins from wheat. After hydrolysis with hydrochloric acid, glutamic acid is removed as its hydrochloride by saturation with hydrochloric acid gas.

* *Ber.*, 1899, **32**, 3191. † *Gazz.*, 1887, **17**, 519.

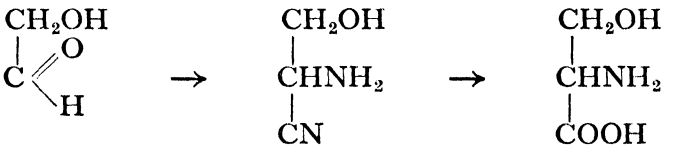
Wolff* synthesized glutamic acid by the reduction of α-isonitrosoglutaric acid.

Hydroxy- and Thio-amino Acids

Serine. — This hydroxyamino acid was obtained by Cramer as early as 1865 among the products resulting from the hydrolysis of silk with sulphuric acid. Since that time it has frequently been encountered among the hydrolytic products of the proteins. The natural form is lævorotatory.

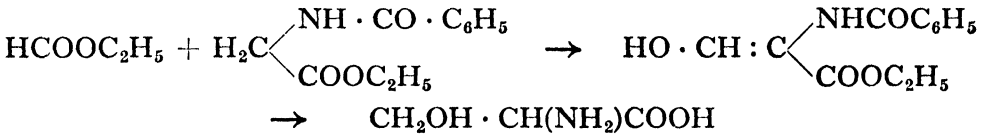
When treated with nitrous acid it is transformed into glyceric acid, while with hydriodic acid it is reduced to alanine. The following are the more important methods by which the acid has been synthesized.

(1) Fischer and Leuchs † obtained a small yield of serine by the hydrolysis of the aminocyanhydrin of glycollic acid:

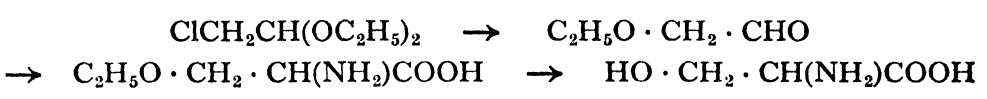


Fischer and Jacobs ‡ resolved the synthetic acid by the fractionation of the brucine or quinine salt of the *p*-nitrobenzoyl derivative.

(2) Erlenmeyer § condensed formic ester with hippuric ester in the presence of sodium ethoxide and reduced the resulting product with aluminium-mercury couple:

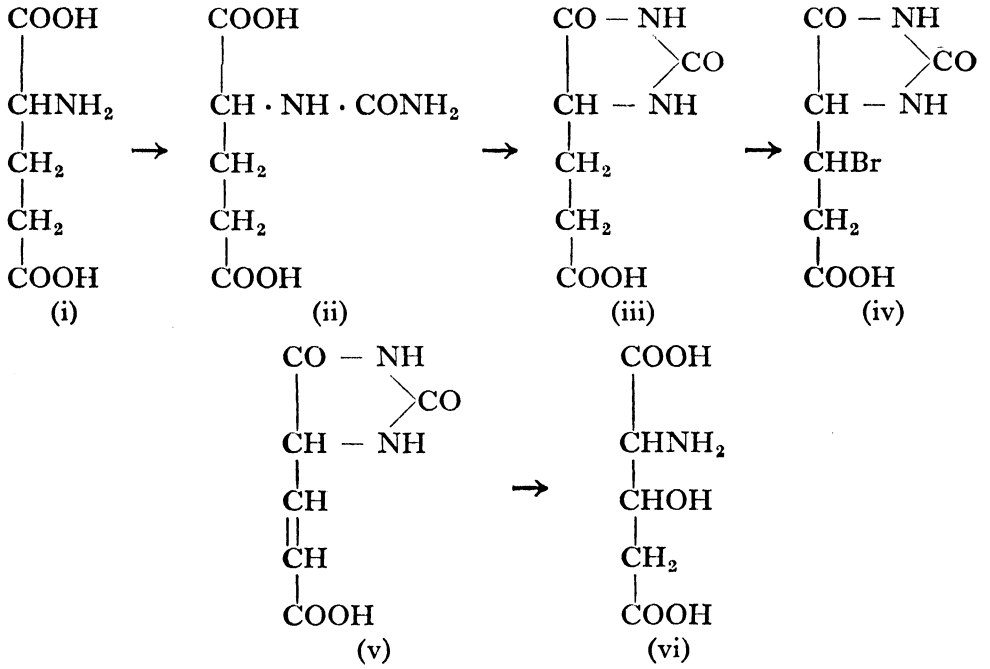


(3) Leuchs and Geiger || synthesized the acid from ethoxyacetal by Strecker's method, subsequently removing the ethyl group with hydrobromic acid. Ethoxyacetal was prepared from chloracetal by the action of sodium ethoxide.



* *Ann.*, 1890, **260**, 79. † *Ber.*, 1902, **35**, 3787; 1906, **39**, 2942; 1907, **40**, 1501.
‡ *Ber.*, 1906, **39**, 2948. § *Ber.*, 1902, **35**, 3769. || *Ber.*, 1906, **39**, 2644.

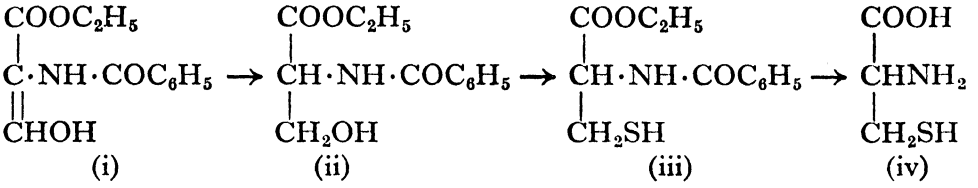
β-Hydroxyglutamic Acid.—As already mentioned, this acid was obtained by Dakin * by extraction of the hydrolytic products of casein with butyl alcohol. More recently it has been identified among the hydrolytic products of glutenin and gliadin.† Its synthesis appears to have presented considerable difficulty, but was eventually achieved from glutamic acid as follows. Glutamic acid (i) was converted into α-uraminoglutaric acid (ii) by the action of potassium cyanate, and hydantoinpropionic acid (iii) obtained from the latter by warming with hydrochloric acid. On treatment with bromine in glacial acetic acid, hydantoin β-bromopropionic acid (iv) was formed, which, on boiling with water, gave hydantoinacrylic acid (v). On prolonged boiling with barium hydroxide solution β-hydroxyglutamic acid (vi) was obtained:



Cysteine and Cystine.—These two compounds embrace the greater part of the sulphur obtained by the hydrolysis of the proteins. Cysteine is the sulphur analogue of serine, while cystine may be regarded as the disulphide. Cystine may be reduced to cysteine by the action of zinc and dilute sulphuric acid, while the reverse change may be brought about by exposing an ammoniacal solution of cystine to the atmosphere. Cysteine may be readily prepared from hair. It has been obtained synthetically by Erlenmeyer junior‡ as follows:

* *Biochem. J.*, 1918, **12**, 290. † Dakin, *Biochem. J.*, 1919, **13**, 398.
‡ *Ann.*, 1904, **307**, 236.

Ethylformylhippurate (i), obtained by the condensation of formic and hippuric esters, is reduced to the ester of benzoylserine (ii). By the action of phosphorus pentasulphide a thio derivative (iii) is obtained which on hydrolysis gives racemic cysteine (iv).



(i) (ii) (iii) (iv)

Cysteine has been obtained from *l*-serine * by converting it, with the aid of phosphorus pentachloride, into β-chloro-α-aminopropionic acid and then treating the latter with barium hydrosulphide:

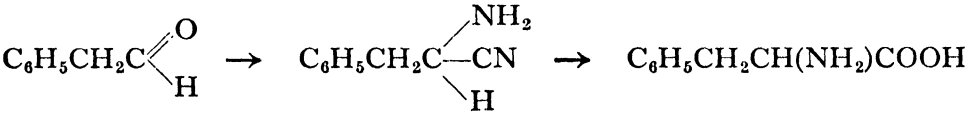
$$CH_2OH \cdot CH(NH_2)COOH \rightarrow CH_2Cl \cdot CH(NH_2)COOH \rightarrow CH_2SH \cdot CH(NH_2)COOH$$

Cystine sometimes separates from urine as a sediment and is also a component of some gall-stones. Mercaptans, sulphides, and substituted sulphuric acids are obtained by the decomposition of these sulphur compounds by living organisms.

The Aromatic Amino Acids

Phenylalanine (α-amino-β-phenylpropionic acid) was found by Schultze † in plant seedlings and in the products of hydrolysis of seed proteins. Since that time it has been shown to be a constituent of many proteins.

This amino acid was first synthesized by Erlenmeyer and Lipp ‡ by the application of the cyanhydrin reaction to phenylacetaldehyde:



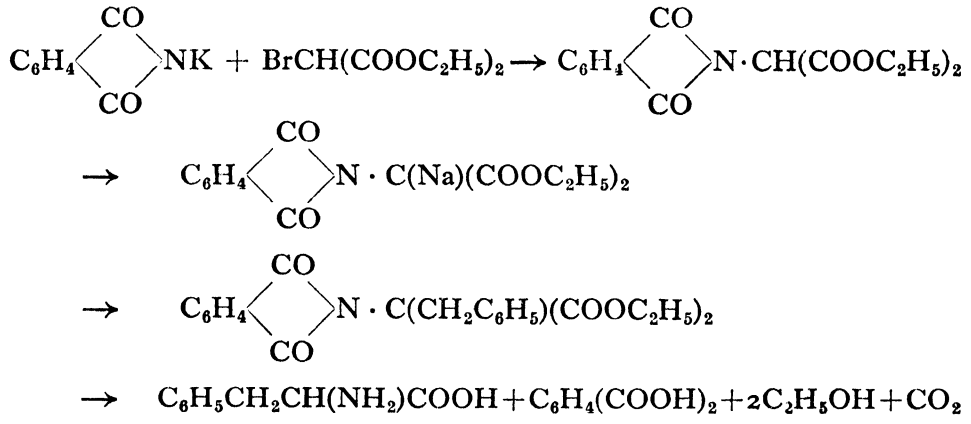
Fischer § synthesized the acid starting from benzylmalonic acid, which was obtained from benzylchloride and the sodium derivative of malonic ester:

$$C_6H_5CH_2Cl + NaCH(COOC_2H_5)_2 \rightarrow C_6H_5CH_2CH(COOC_2H_5)_2 \rightarrow C_6H_5CH_2 \cdot CH(COOH)_2 \rightarrow C_6H_5CH_2CBr(COOH)_2 \rightarrow C_6H_5CH_2CHBrCOOH \rightarrow C_6H_5CH_2CH(NH_2)COOH$$

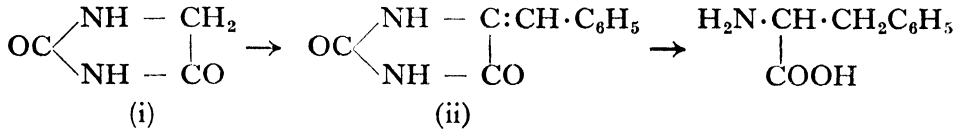
* Fischer and Raske, *Ber.*, 1908, **41**, 893.
† *Ber.*, 1881, **14**, 1785; *Zeit. physiol. Chem.*, 1884, **9**, 63.
‡ *Ber.*, 1882, **15**, 1006. § *Ber.*, 1900, **33**, 2383; 1904, **37**, 3064.

Fischer subsequently resolved the acid into its optical isomers.

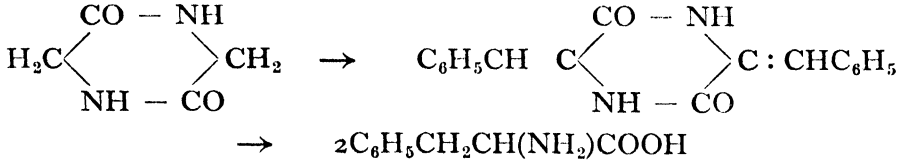
Sörensen * synthesized phenylalanine by the aid of the phthalimide reaction:



Wheeler and Hoffmann † condensed benzaldehyde with hydantoin (i) and the resulting benzylidene hydantoin (ii) on treatment with hydriodic acid gave phenylalanine:



More recently Sasaki ‡ has obtained it by the reduction and hydrolysis of the product obtained by the condensation of benzaldehyde and diketopiperazine in the presence of sodium acetate and acetic anhydride:



Tyrosine and dihydroxyphenylalanine were obtained in a similar way.

Tyrosine (*β*-*p*-hydroxyphenyl-*α*-aminopropionic acid) was obtained by Liebig in 1846 by heating cheese (*τυρός*) with caustic potash. It is the least soluble of the amino acids, and is therefore easily isolated from the hydrolytic products of the proteins. It was first synthesized by Erlenmeyer and Lipp § by nitrating phenyl-

* *Zeit. physiol. Chem.*, 1905, **44**, 448.
† *Amer. Chem. J.*, 1911, **45**, 368.
‡ *Ber.*, 1921, **54** [B], 163, 2056.
§ *Ann.*, 1883, **219**, 161, 179.

alanine, reducing the resulting para-nitro derivative, and then replacing the amino group by hydroxyl by means of nitrous acid:

$$C_6H_5CH_2CH(NH_2)COOH \rightarrow O_2N \cdot C_6H_4CH_2CH(NH_2)COOH \rightarrow H_2N \cdot C_6H_4CH_2CH(NH_2)COOH \rightarrow HO \cdot C_6H_4 \cdot CH_2 \cdot CH(NH_2)COOH$$

Erlenmeyer junior and Halsey * obtained tyrosine from the condensation product of *p*-hydroxybenzaldehyde with hippuric acid in the presence of sodium acetate and acetic anhydride:

$$HO \cdot C_6H_4CHO + \begin{matrix} CH_2COOH \\ | \\ NH \cdot COC_6H_5 \end{matrix} \rightarrow \begin{matrix} HO \cdot C_6H_4 \cdot CH : C \cdot CO \\ \qquad\qquad\quad \diagdown\diagup \\ \qquad\qquad\quad N \cdot COC_6H_5 \end{matrix}$$

$$\rightarrow \begin{matrix} HO \cdot C_6H_4CH : C \cdot COOH \\ \qquad\quad | \\ \qquad\quad NH \cdot CO \cdot C_6H_5 \end{matrix} \rightarrow \begin{matrix} HO \cdot C_6H_4 \cdot CH_2 \cdot CH \cdot COOH \\ \qquad\qquad\quad | \\ \qquad\qquad\quad NH \cdot CO \cdot C_6H_5 \end{matrix}$$

$$\rightarrow \begin{matrix} HO \cdot C_6H_4 \cdot CH_2 \cdot CH \cdot COOH \\ \qquad\qquad\quad | \\ \qquad\qquad\quad NH_2 \end{matrix} + C_6H_5COOH$$

Wheeler and Hoffmann † condensed anisaldehyde with hydantoin and treated the resulting product with hydriodic acid. The latter brings about reduction and hydrolysis, and at the same time removes the methyl group from the methoxy group.

$$\begin{matrix} OC\diagup NH - C : CH \cdot C_6H_4OCH_3 \\ \qquad\diagdown NH - CO \end{matrix} \rightarrow \begin{matrix} H_2N \cdot CH \cdot CH_2 \cdot C_6H_4OH \\ \qquad\quad | \\ \qquad\quad COOH \end{matrix}$$

Heterocyclic Amino Acids

Proline (α-pyrrolidine carboxylic acid).—This amino acid was discovered by Fischer in 1901 among the products of hydrolysis of casein. It has also been obtained by the hydrolysis of a number of proteins of vegetable origin, notably the prolamines, but it has not yet been found to occur as such in any plant. It is readily soluble in alcohol, and can be partially separated from other amino acids by this solvent.

The racemic form was synthesized by Willstätter ‡ by the action of ammonia on αδ-dibromvaleric acid:

$$\begin{matrix} CH_2 - CH_2 \\ | \quad\quad | \\ CH_2 \quad CH \cdot COOH \\ \diagdown\quad\diagup \\ \quad Br \quad Br \end{matrix} + NH_3 = \begin{matrix} CH_2 - CH_2 \\ | \quad\quad | \\ CH_2 \quad CH \cdot COOH \\ \diagdown\quad\diagup \\ \quad\ NH \end{matrix} + 2HBr$$

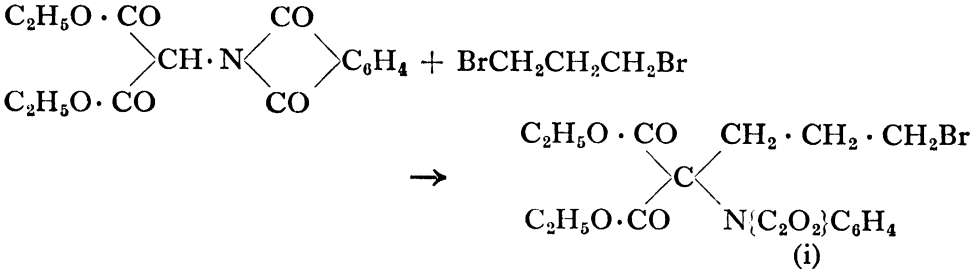
* *Ber.*, 1897, **30**, 2981; *Ann.*, 1899, **307**, 138. † *Amer. Chem. J.*, 1911, **45**, 368.
‡ *Ber.*, 1900, **33**, 1162; *Ann.*, 1902, **326**, 94, 104.

The αδ-dibromvaleric acid was prepared from trimethylene dibromide and sodium malonic ester as follows:

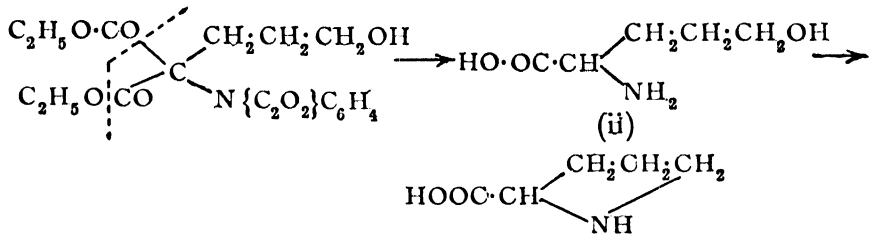
$$Br\cdot CH_2\cdot CH_2\cdot CH_2\cdot Br + NaCH(COOC_2H_5)_2 \rightarrow Br[CH_2]_3CH(COOC_2H_5)_2$$

$$\xrightarrow{Br_2} Br[CH_2]_3C\cdot Br(COOC_2H_5)_2 \xrightarrow{HBr} Br[CH_2]_3CHBrCOOH$$

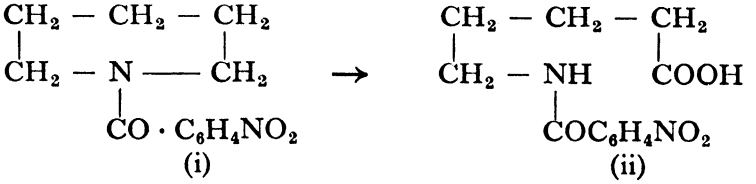
Fischer and Zemplen,* and also Sörensen and Anderson,† have also synthesized the acid by the application of the phthalimide-malonic ester method: Phthaliminomalonic ester and trimethylene dibromide are condensed to give γ-bromopropylphthalimino-malonic ester (i):



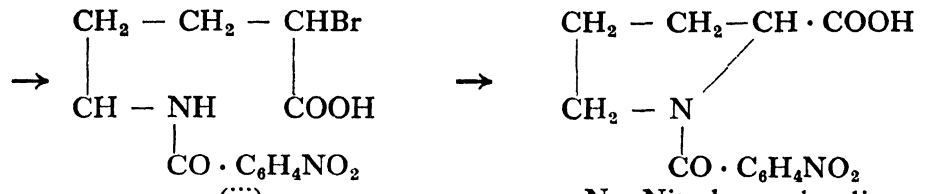
and the bromine atom replaced by hydroxyl by the action of alcoholic caustic soda, after which hydrochloric acid converts the product into α-amino-δ-hydroxyvaleric acid (ii). On evaporation with hydrochloric acid, *dl*-proline is obtained



Optically active proline was obtained synthetically as follows:‡ N*m*-nitrobenzoylpiperidine (i) was oxidized to δ[-*m*-nitrobenzoyl-amino]-valeric acid (ii) by the action of potassium permanganate. This product was then brominated, and the bromo derivative (iii) transformed into N*m*-nitrobenzoylproline by the action of alkali:



* *Ber.*, 1909, **42**, 1022. † *Zeit. physiol. Chem.*, 1908, **56**, 236.
‡ *Ber.*, 1911, **44**, 1332.



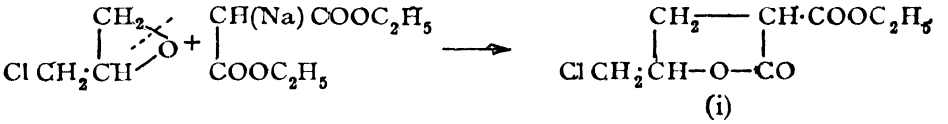
(iii)

Nm-Nitrobenzoylproline.

This compound was then resolved into its optical isomerides by fractionally crystallizing its cinchonine salts, after which the active nitrobenzoylproline was converted into nitrobenzoic acid and the active proline by the action of hydrochloric acid.

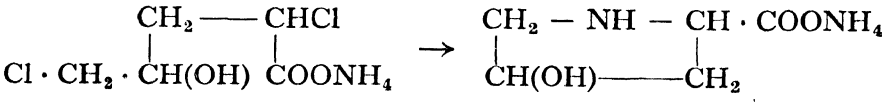
Hydroxyproline (β'-Hydroxypyrrolidine-α-carboxylic acid).—This amino acid was discovered by Fischer among the products of hydrolysis of gelatine. Its isolation presented considerable difficulty, but in recent years it has been obtained from several proteins.

This amino acid contains two asymmetric carbon atoms, and all four stereoisomeric forms have been recently obtained by Leuchs.* The two inactive forms were synthesized as follows: † δ-chloro-γ-valerolactone-α-carboxylic ester (i) was obtained by the condensation of epichlorhydrin with sodium malonic ester:

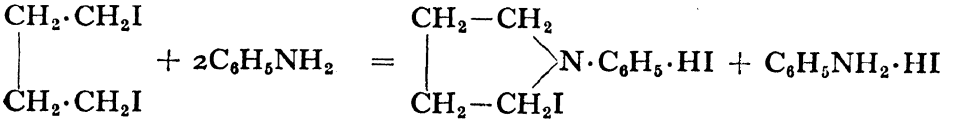


(i)

After chlorination and saponification with concentrated hydrochloric acid, the lactone ring was opened by treatment with ammonia and the ammonium salt of hydroxyproline formed by immediate ring formation:



The last stage of this reaction is analogous to the reactions whereby 1 : 4-dihalogen paraffins are transformed by the action of primary amines in alcoholic solution into N-alkyl- and N-aryl-pyrrolidines,‡ e.g.

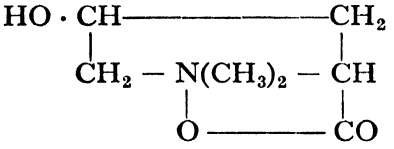


When treated with methyliodide and methylalcoholic potash,

* *Ber.*, 1919, 52 [B], 2086. † *Ber.*, 1907, 40, 30.

‡ Scholtz, *Ber.*, 1899, 32, 848; V. Braun, *Ber.*, 1911, 44, 1254.

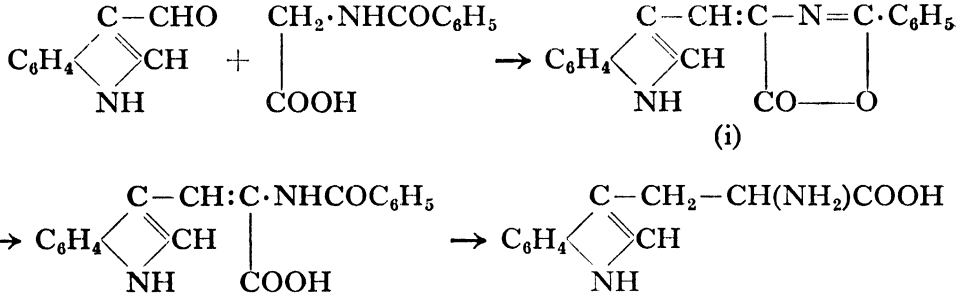
hydroxyproline gives a mixture of two stereoisomeric oxyproline dimethylbetaines (hydroxystachydrines) * (p. 216).



Tryptophane (Indole-α-aminopropionic acid). — This interesting amino acid was first obtained in a pure crystalline condition, from casein, by Hopkins and Cole † in 1901. The natural form is lævorotatory, and it is present in nearly all proteins. It is, however, entirely absent from zein, the prolamine of maize, and it is also absent from gelatine.

On fusion with potash it yields skatole and indole (p. 182). In the presence of putrefactive bacteria, indole acetic acid and indole propionic acid are formed in addition.

Tryptophane has been obtained synthetically by Ellinger and Flamand ‡ as follows: Indole-β-aldehyde is condensed with hippuric acid in the presence of sodium acetate and acetic anhydride to give the lactone (i). On hydrolysis with boiling sodium hydroxide solution and subsequent reduction with sodium in alcohol, tryptophane is obtained:



Indole-β-aldehyde was obtained by the action of chloroform and caustic potash on indole (Reimer's reaction).

Histidine (α-amino-β-iminazolepropionic acid).—This amino acid was first discovered by Kossel § among the decomposition products of the protein sturine, which was obtained from the spermatazoa of the sturgeon. From some proteins such as globin the yield may be as high as 10 per cent, and it is conveniently prepared

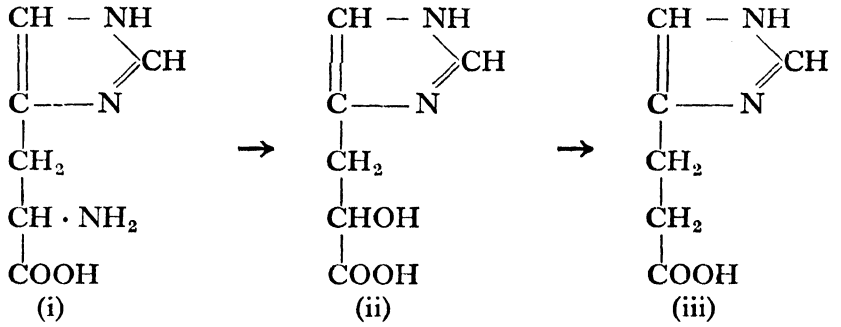
* Schultze, Trier, *Zeit. physiol. Chem.*, 1912, **79**, 240; Küng, *ibid.*, 1913, **85**, 217. † *Journ. Physiol.*, 1901, **27**, 418.

‡ *Ber.*, 1907, **40**, 3029; *Zeit. physiol. Chem.*, 1908, **55**, 8.

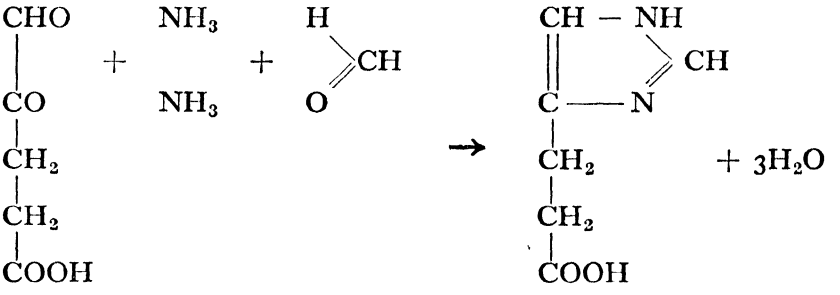
§ *Zeit. physiol. Chem.*, 1896, **23**, 176.

from ox blood. When treated with alkaline solutions of diazonium salts it forms a red-coloured product, and this is in accordance with its constitution as an iminazole derivative.

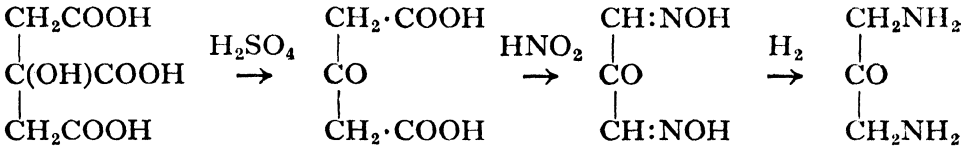
Knoop and Windaus * observed that when histidine (i) is treated with nitrous acid, it gives a product (ii) which on reduction yields β-iminazolepropionic acid (iii):



The same authors have synthesized histidine by the combined action of ammonia and formaldehyde on glyoxylpropionic acid:

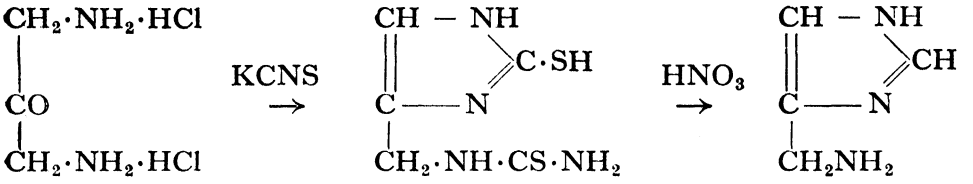


The constitutional formula of histidine has been fully confirmed by its synthesis by Pyman by two independent methods. According to the first method,† citric acid is converted by well-known methods successively into diaminoacetone:

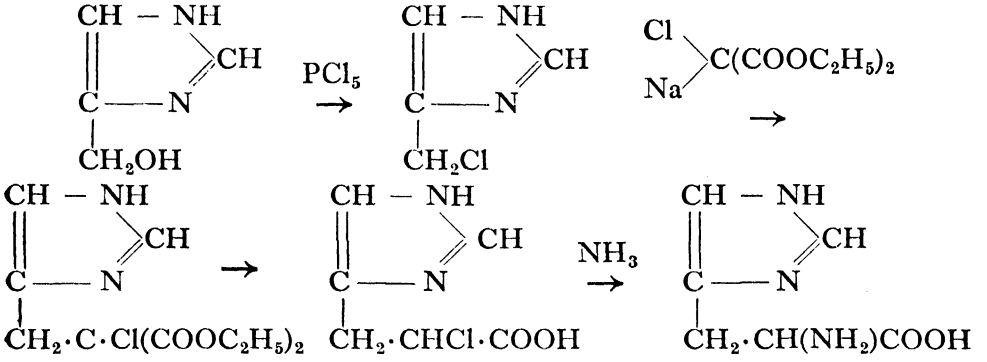


Gabriel's method of synthesizing an iminazole ring, which consists in acting on an aminoketone with potassium thiocyanate and oxidizing the product with nitric acid, whereby the thiol group (SH) is removed, was next employed:

* *Beitr. z. Chem. Phys. u. Path.*, 1905, 7, 144.
† Pyman, *Trans.*, 1911, 99, 672, 1392, 2172.

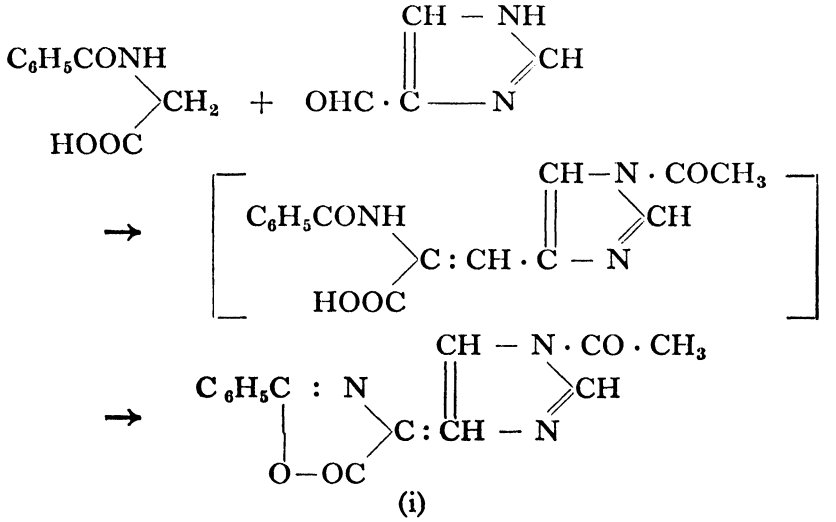


The product was then treated with nitrous acid, which replaces the amino group by hydroxyl, the hydroxyl substituted by chlorine, and the product condensed with sodium chloromalonic ester. The resulting product was hydrolyzed, carbon dioxide removed, and the chlorine atom replaced by an amino group:



The racemic product thus obtained was resolved by the fractional crystallization of its tartrate, when the lævo compound was found to be identical with the natural substance.

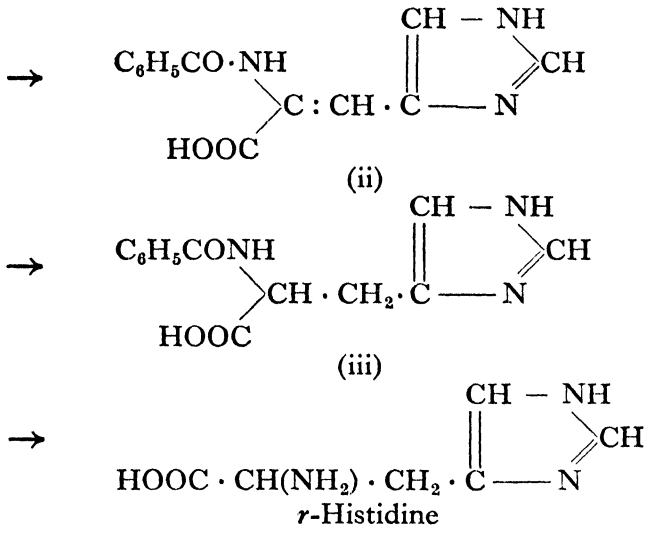
According to the second method,* glyoxaline-4 (or 5)-formaldehyde is condensed with hippuric acid according to Erlenmeyer's method with the production of the lactone (i):



(i)

* Pyman, *Trans.*, 1916, **109**, 186.

On boiling with dilute sodium carbonate the acetyl group is removed, the oxazoline ring opened, and α-benzoylaminoglyoxaline-4 (or 5)-acrylic acid (ii) obtained. On reduction, benzoylhistidine (iii) is obtained which gives racemic histidine on hydrolysis:



r-Histidine

The Distribution of Amino Acids in the Proteins.—Following the isolation and characterization of the various amino acids present in the proteins, chemists began to consider the losses of amino acids which occur in their separation, with a view to arriving at quantitative results for the distribution of these acids in the various proteins. This line of inquiry has been vigorously pursued by Abderhalden, who has ascertained the component amino acids of the albumins of egg, serum, and milk, as well as other proteins.

Osborne and his collaborators have investigated gliadin (from gluten of wheat and rye), hordein (from barley), zein (from maize), and several allied proteins.

In 1907 Fischer investigated the fibroin produced by silkworms and spiders, incidentally emphasizing the remarkable biological fact that there is only slight chemical difference between the synthetic products of two creatures whose diet is so vastly divergent. The principal difference is the large proportion of glutamic acid which has been derived from ordinary silk, and the absence of serine.

More recently Foreman * has applied his method for the isolation of the amino acids to caseinogen with even more satisfactory results.

The attached tabulation illustrates a few of the results obtained in this direction.

* *Biochem. J.*, 1919, **13**, 378.

AMINO ACIDS IN VARIOUS PROTEINS

Protein.	Salmine.	Globin of Hæmoglobin, Dog's Blood.	Egg Albumin.	Edestin from Hemp.	Caseinogen from Cow's Milk.	Silk Fibroin of Spider.	Hæmoglobin.
Class of Protein.	Protamine.	Histone.	Albumins.	Globulin.	Phosphoprotein.	Scleroprotein.	Chromoprotein.
Glycine	—	+	—	3·8	0	35·2	0
Alanine	+	3·0	2·2	3·6	1·5	23·4	4·19
Valine	4·3	1·0	2·5	+	7·2	—	—
Leucine, isoleucine	+	17·5	10·7	20·9	9·4	1·8	30·0
Aspartic acid ..	—	2·5	2·2	4·25	1·4	—	4·43
Glutamic acid ..	—	1·2	9·1	6·3	15·6	11·7	1·73
Serine	7·8	—	—	0·33	0·5	—	0·56
Cystine	—	—	—	0·25	?	—	0·31
Lysine	0	—	3·7	1·0	6·0	5·2	4·28
Arginine	87·4	—	4·9	11·7	3·8	—	5·4
Proline	11·0	4·5	3·5	4·1	6·7	3·7	2·34
Hydroxyproline ..	—	—	—	2·0	0·3	—	1·04
Histidine	0	—	1·7	1·1	2·5	—	10·96
Tryptophane ..	—	—	+	+	1·5	—	+
Phenylalanine ..	—	5·0	5·0	3·1	3·2	—	4·24
Tyrosine	—	—	1·7	2·11	4·5	8·2	1·33
Total	110·5	34·7	47·2	64·54	64·1	89·2	70·81

THE POLYPEPTIDES

After his researches on the amino acids, commenced in 1899, had given some indication of the nature and variety of these chemical units, Fischer next turned his attention to the artificial elaboration of the protein molecules from their components. The amount of nitrogen liberated from the proteins by nitrous acid is small in comparison with the percentage of nitrogen in the original molecule. This fact, in conjunction with the early recognition of hippuric acid as benzoyl glycine, gave a clue as to the way in which the amino acids are linked together. According to the number of amino acid groups present in the molecule, the synthetic products were termed di-, tri-, &c., peptides. The simplest is the dipeptide, glycyl-glycine, formed by the union of two molecules of glycine:

$$NH_2CH_2COOH + NH_2CH_2COOH = NH_2CH_2CO\cdot NHCH_2COOH + H_2O$$

Glycylglycine

Synthesis of the Polypeptides.—As early as 1882, Curtius obtained two acids by the action of benzoyl chloride on the silver

salt of glycine. One of these compounds had twice the molecular weight expected, and was shown to be hippurylamidoacetic acid:

$$C_6H_5CO \cdot NH \cdot CH_2CO \cdot NH \cdot CH_2COOH$$

In 1904 the same chemist made the first systematic attempt * to link together a series of amino acids in chains. For this purpose glycine ester, quite free from its hydrochloride, was dissolved in dry ether and allowed to stand, when triglycylglycine ester (i) was slowly deposited:

$$NH_2CH_2CO[NHCH_2CO]_2NH \cdot CH_2COOC_2H_5$$
(i)

The use of the azoimides effected a considerable improvement, e.g. benzoyl azoimide combines with glycine with the formation of hippuric acid and the removal of hydrazoic acid (azoimide):

$$C_6H_5CO \cdot N\langle \overset{N}{\underset{N}{\|}} + H_2N \cdot CH_2 \cdot COOH = C_6H_5CO \cdot NH \cdot CH_2COOH + HN_3$$

The ester of hippuric acid may in turn be converted into the azoimide and combined with a second molecule of glycine:

$$C_6H_5CO \cdot NH \cdot CH_2CON_3 + H_2N \cdot CH_2COOH = C_6H_5CONHCH_2CONH \cdot CH_2COOH + N_3H$$

In this way chains of different amino acids were obtained, but the method suffered from the drawback that the benzoyl group could not be eliminated without complete hydrolysis to the constituent amino acids.

The following methods, which were devised by E. Fischer, are much more satisfactory.

1. *The action of acids or alkalies upon derivatives of 2 : 5-diketopiperazine.*

It is well known that amino acid esters are changed by heat into derivatives of 2:5-diketopiperazine, and these compounds yield dipeptides on partial hydrolysis:

$$\begin{array}{l} CH_2 - CO \\ NH\langle \qquad \rangle NH \\ CO - CH_2 \end{array} + H_2O = H_2N \cdot CH_2 \cdot CO \cdot NH \cdot CH_2 \cdot COOH$$

* *J. pr. Chem.*, 1904 (2), **70**, 57.

2. *By the action of ammonia on the condensation product of the acid chlorides of halogen fatty acids and the amino acids or their esters.*

This method makes possible the successive introduction of different amino acid radicles into a simple polypeptide or amino acid. The following synthesis of leucylglycylglycine through the intermediate glycylglycine is quite straightforward:

$$CH_2ClCOCl + H_2N\cdot CH_2\cdot COOH \rightarrow CH_2ClCO\cdot NH\cdot CH_2COOH$$
$$(+ NH_3) \rightarrow CH_2NH_2CONHCH_2COOH$$

$$\begin{matrix} CH_3 \\ \\ CH_3 \end{matrix}\!\!\rangle CH\cdot CH_2CHBrCOCl + H_2N\cdot CH_2CO\cdot NHCH_2COOH$$

$$\rightarrow \begin{matrix} CH_3 \\ \\ CH_3 \end{matrix}\!\!\rangle CH\cdot CH_2\cdot CHBrCO\cdot NH\cdot CH_2\cdot CO\cdot NHCH_2COOH$$

$$\rightarrow \begin{matrix} CH_3 \\ \\ CH_3 \end{matrix}\!\!\rangle CH\cdot CH_2\cdot CH(NH_2)CO\cdot NHCH_2CO\cdot NHCH_2COOH$$

By using the halogen acid chlorides of optically active acids, optically active polypeptides are obtained. The method only allows of the amide groups being introduced into the amino group of the original acid, so that the chain can be lengthened only at this end.

3. *From the acid chlorides of amino acids.*

This complementary method arose as a result of the observation in 1904 that the chlorides of halogenated arylamino acids may be prepared by the action of phosphorus pentachloride on the acid dissolved in acetyl chloride. These compounds react with the esters of amino acids and polypeptides, and after hydrolysis of the product the halogen is replaced by ammonia. In this way several higher polypeptides have been prepared, e.g.

$$C_4H_9CHBrCONHCH_2COCl + NH_2CH_2CONHCH_2COOC_2H_5$$
Bromisocapronylglycyl chloride — Glycylglycine ester.

$$\rightarrow C_4H_5CHBrCONHCH_2CONHCH_2CONHCH_2COOC_2H_5$$

$$\rightarrow C_4H_9CH(NH_2)CO[NHCH_2CO]_2NH\cdot CH_2COOH$$
Leucyldiglycyl glycine

The acid chlorides may be obtained by the action of thionyl chloride on the amino acids in which the amino group has been protected by carbomethoxylation (p. 86), e.g. carbethoxyglycyl chloride and glycine ester give carbethoxylglycylglycine ester, from which the

amide can be obtained by hydrolysis; but the carbethoxyl group cannot be removed without complete hydrolysis of the molecule into its constituent units:

$$(C_2H_5OOC)NH \cdot CH_2 \cdot COCl + NH_2CH_2COOC_2H_5 \rightarrow (C_2H_5OOC) \cdot NH \cdot CH_2CONH \cdot CH_2 \cdot COOC_2H_5$$

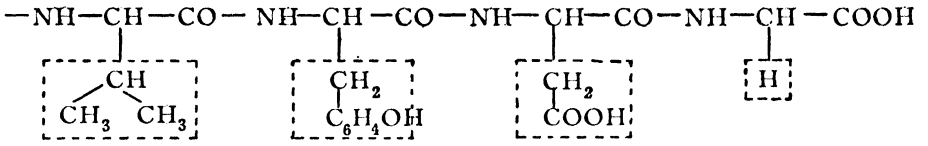
Carbethoxylglycylglycine ester

Although this process is elastic, the solubility of many such acyl chlorides in acetyl chloride, and the consequent difficulty of separating them from solution without decomposition, presented a serious obstacle to its extension. This was overcome by preparing the acyl chlorides of the amino acid hydrochlorides themselves. These chlorides, having the general formula $[R \cdot C \cdot H(NH_3Cl)COCl]$, are also substituted ammonium chlorides, and are generally not readily soluble in acetyl chloride. As they act smoothly on the esters of amino acids and polypeptides, the device has been a most fruitful one, and particularly useful in its application to the *d*- and *l*-amino acids, with consequent synthesis of optically active polypeptides.

Straightforward as these reactions appear in description, they represent a very remarkable experimental feat, the rigid exclusion of water being necessary throughout.

The attached tabulation (p. 168) briefly illustrates the variety of polypeptides prepared by these reactions, but it only embraces a few of the numerous products obtained.*

The Relation of the Polypeptides to the Simpler Proteins.—It is generally believed that the amino acids are linked together in the protein molecules as in the polypeptides, i.e. the amino group of one molecule is linked to the carboxyl group of its neighbouring amino acid to form long chains, as for example:



Valyl residue | Tyrosine residue | Aspartic acid residue | Glycine residue

It is obvious that the field of investigation is an extremely wide one, and an interesting calculation of the possibilities presenting themselves among the polypeptides has been made by Fischer.

* For further examples see Emil Fischer's lecture (*Ber.*, 1906, 39, 551).

SOME POLYPEPTIDES SYNTHESIZED BY FISCHER'S METHODS

By the Action of	On	Product.	Reference.
Hydrochloric acid.	Glycine anhydride.	Glycylglycine.	*Ber.*, 1901, 34, 2890.
Ammonia.	Chloracetylalanine.	Glycyl-*dl*-alanine.	*Ber.*, 1904, 37, 2489.
Ammonia.	Brompropionyl-glycine.	*dl*-Alanylglycine.	*Ann.*, 1905, 340, 130.
Ammonia.	*d*-Bromisocaproyl. *d*-Alanine.	*l*-Leucyl. *d*-Alanine.	*Ber.*, 1906, 39, 2916.
Phenylalanyl chloride.	Glycine ester.	Phenylalanylglycine.	*Ber.*, 1905, 38, 2919.
d-Tryptophyl chloride.	Glycine ester.	*d*-Tryptophylglycine.	*Ber.*, 1907, 40, 2741.
Ammonia.	Chloracetylglycyl-glycine.	Diglycylglycine.	*Ber.*, 1903, 36, 2983. *Ber.*, 1904, 37, 2500.
Liquid ammonia.	Bromisocapronyl-octaglycylglycine.	Leucyloctaglycyl-glycine.	*Ber.*, 1906, 39, 2906.

According to this estimate, the octadecapeptide has 816 possible isomerides, while a polypeptide comprising 30 amino acids—of which 5 are glycine, 4 alanine, 3 leucine, 3 lysine, 2 tyrosine, 2 phenylalanine, and 13 various other amino acids—has a number of possible isomerides reaching $1{\cdot}28 \times 10^{27}$. In these calculations it is assumed that the mechanism of linking the amino acid groups is limited to that of glycylglycine, and further complexity would arise from alternative linkages such as that of diketopiperazine. Moreover, hydroxyamino acids may participate in the linkages peculiar to esters and ethers.

The far-reaching consequences of the methods provided to separate the components of an amino acid mixture have already been indicated, but the esters thus isolated were, until 1902, those of amino acids only, unassociated with polypeptides. In that year Fischer and Bergell produced from silk fibroin, by successive hydrolysis with hydrochloric acid, trypsin, and baryta, a dipeptide which appeared to be glycyl-*d*-alanine, although it could not be identified with the synthetic product; but in 1906 Fischer and Abderhalden obtained from the same source a methyldiketopiperazine, identical with that producible from glycine and *d*-alanine, thus indicating

that glycyl-*d*-alanine is amongst the degradation products of silk fibroin. Soon after this the following polypeptides were recognized: glycyl-*d*-tyrosine (silk fibroin), glycyl-*l*-leucine and *d*-alanyl-*l*-leucine (elastin), *l*-leucyl-*d*-glutamic acid (gliadin), glycyl-*d*-alanyl-glycyl-*l*-tyrosine (silk fibroin), and glycylproline anhydride (gelatine).

As early as 1888 De Rey-Pailhade showed that extracts of yeast and many animal tissues are able to reduce sulphur to hydrogen sulphide. Quite recently Hopkins has shown that this is due to the presence of a dipeptide of cysteine and glutamic acid, which is provisionally termed " glutathione ". This compound is not affected by proteolytic enzymes of the tissues, but is hydrolyzed by boiling acids to equivalent proportions of cystine and glutamic acid. Glutathione is an autoxidizable substance, and is of exceptional importance in relation to the oxidation and reduction processes which take place in living cells. In neutral or slightly alkaline solution it is oxidized spontaneously to the disulphide and acts as oxygen acceptor, while the oxidized form, on the other hand, acts as a hydrogen acceptor. This dipeptide is formed by the union of an amino group of one acid with a carboxyl group of the other, with elimination of water, but the exact allocation of the union is not yet known.*

Although the aggregate number of synthetic polypeptides must exceed two hundred, the study of these compounds, demanding an experimental technique of the highest order, has served but partially to illuminate the gulf which still separates the chemist from his goal in the study of the proteins.

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* *Biochem. J.*, 1921, **15**, 286. For further information on the significance of this compound the reader should consult, *Oxidations and Reductions in the Animal Body*, by Dakin, 2nd Edition, London, 1922.

CHAPTER VIII

Some Simple Natural Organic Bases

Introduction and Scope.—The classification of the natural organic bases is a matter of considerable difficulty. Many of the familiar complex nitrogenous bases, now classified as alkaloids, were discovered long before organic chemistry had become a systematic science; indeed, as early as 1806 Sertürner had discovered the basic nature of morphine, and in the next few years a large number of plant bases, including narcotine, strychnine, brucine, caffeine, and quinine, were isolated.

These bases are almost insoluble in water and may be readily extracted with the aid of immiscible solvents. This fact, together with their pronounced physiological activity, enhanced the study of the vegetable alkaloids.

As a rule the animal bases are readily soluble in water, and cannot be conveniently extracted with immiscible solvents. As a consequence of their isolation requiring a special technique, very few of the animal bases were isolated before 1890. The first great advance was made in 1885 by Brieger, who introduced precipitation methods whereby he isolated putrescene, cadaverine, and several other putrefaction bases.

In this book the natural organic bases will be considered in three chapters:

1. Bases derived from amino acids and other simple natural bases.
2. The pyrimidine and purine bases.
3. The alkaloids.

This classification is largely one of convenience, and it should be remembered that no rigid classification of the natural organic bases is yet practicable.

Occurrence and Isolation of the Simple Natural Bases.—Having excluded the alkaloids and the pyrimidine and purine bases

from the scope of the present chapter, we may briefly consider the occurrence and isolation of the other natural bases.

The first stage of putrefaction is the hydrolysis of proteins into their constituent amino acids, but bacteria are able to break down amino acids still further. This degradation may take place in two ways: either an amino group may be eliminated (deaminization) or a carboxyl group may be removed (decarboxylation). In the first portion of this chapter the amines derived by the decarboxylation of monobasic amino acids will be dealt with. Decarboxylation may take place either by the simple removal of carbon dioxide, or the carboxyl group may be eliminated as formic acid, in which case reduction must take place:

$$R{\cdot}CH{\cdot}NH_2{\cdot}COOH \longrightarrow R{\cdot}CH_2{\cdot}NH_2 + CO_2$$

$$R{\cdot}CH{\cdot}NH_2{\cdot}COOH \longrightarrow R{\cdot}CH_2{\cdot}NH_2 + H{\cdot}COOH$$
$$\quad H \qquad\quad H$$

The same process applied to dibasic monoamino acids results in the production of ω-amino acids, and as these substances still contain a carboxyl group they are only feeble bases. During the last few years almost all the amino acids have been converted into the corresponding bases either by bacteria or by chemical means.

It has already been stated (p. 103) that when animal and vegetable tissues are extracted with ether, in addition to fats, oils, and cholesterol, small quantities of certain complex substances are extracted which are termed lipins. Of these the best known are lecithin and kephalin, which on hydrolysis give glycerol, fatty acids, and two amino alcohols, choline and amino-ethyl alcohol. Neurine and trimethylamine are secondary decomposition products of choline.

Creatine, creatinine, and other guanidine derivatives are other interesting animal bases, while the betaïnes may be regarded as derived from the amino acids by methylation.

It has already been pointed out that the majority of the bases dealt with in this chapter are soluble in water and are sparingly soluble in ether and chloroform. A few monoamines like methylamine are volatile in steam, but the majority must be isolated by precipitation methods. The preliminary purification of a tissue extract after removal of coagulable protein is best effected by lead acetate or by tannin. In the former case the solution is first treated by normal lead acetate, and then by the basic salt. After this treat-

ment, which removes the proteins and the peptones, the solution is concentrated, when some bases, such as creatine, may separate. The most important precipitant is phosphotungstic acid in the presence of dilute sulphuric acid. Mercuric chloride and silver nitrate are occasionally employed. For the isolation of the individual bases it is necessary to prepare crystalline derivatives. For this purpose the hydrochlorides, nitrates, picrates, platinichlorides, or aurichlorides may be prepared, or the mixture may be benzoylated.

SIMPLE MONOAMINO BASES

Methylamine, CH_3NH_2, the simplest aliphatic base, occurs in Annual and Perennial Dog's Mercury (*Mercurialis annua* and *M. perennis*) and in the root of the Sweet Flag (*Acorus calamus*). It has been frequently encountered as a product of bacterial action, and may be derived from glycine by decarboxylation or, more probably, from choline.

Methylamine may be obtained synthetically by a variety of simple reactions which need not be discussed here.

Trimethylamine, $(CH_3)_3N$, occurs in leaves of the Stinking Goosefoot (*Chenopodium vulvaria*), the Mountain Ash (*Pyrus aucuparia*), and in the flowers of the Hawthorn (*Cratægus Oxyacantha*). It is of common occurrence in putrefaction products and is derived from choline and similar quaternary bases. As early as 1855 Winckler observed the presence of trimethylamine in herring brine. Trimethylamine is usually prepared by the destructive distillation of beet-sugar molasses, and in this case the parent substance is betaïne.

Hofmann's preparation of the methylamines by the action of alcoholic ammonia on methyliodide is well known.

Isoamylamine, $(CH_3)_2 \cdot CH \cdot CH_2 \cdot CH_2 \cdot NH_2$, is probably present in fresh ergot * and is certainly present in putrid meat.† In these cases it is probably derived from leucine by decarboxylation, and it may be prepared by rapidly heating the latter.

$(CH_3)_2 \cdot CH \cdot CH_2 \cdot CH(NH_2)COOH$ Leucine

$(CH_3)_2 \cdot CH \cdot CH_2 \cdot CH_2 \cdot NH_2$ Isoamylamine

The isoamylamine obtained from ergot and during putrefaction processes is probably mixed with 2-methylaminobutane, derived from

* Barger and Dale, *J. Physiol.*, 1909, 38, 343.
† Barger and Walpole, *ibid.*, 1908, 37, 343.

isoleucine, while normal amylamine, derived from norleucine, may also be present:

$(C_2H_5)(CH_3)CH \cdot CH(NH_2)COOH$ Isoleucine

↓

$(C_2H_5)(CH_3) \cdot CH \cdot CH_2(NH_2)$ Methylaminobutane

$CH_3 \cdot [CH_2]_3 \cdot CH(NH_2)COOH$ Norleucine (caprine)

↓

$CH_3[CH_2]_3CH_2NH_2$ Amylamine

β-Phenylethylamine, $C_6H_5CH_2CH_2NH_2$, isolated from putrid gelatine by Nencki in 1876, was one of the earliest putrefaction bases of which the composition was correctly determined. It is derived by the decarboxylation of phenylalanine (p. 145). It is interesting to note that phenylethylalcohol, $C_6H_5CH_2CH_2OH$, occurs in rose oil (p. 139).

Phenylethylamine is easily obtained synthetically by the reduction of benzylcyanide:

$$C_6H_5CH_2CN + 2H_2 = C_6H_5CH_2CH_2NH_2$$

but the maximum yield so far obtained does not exceed 50 per cent.*

***p*-Hydroxyphenylethylamine,** $HO \cdot C_6H_4 \cdot CH_2 \cdot CH_2 \cdot NH_2$, was first obtained by Schmitt and Nasse in 1865, by the decarboxylation of tyrosine (p. 145) by heat. It is the chief pressor constituent of putrid meat † and is present in extracts of ergot.‡ The following are the more important synthetic methods by which this base has been prepared.

1. The reduction of *p*-hydroxyphenylacetonitrile with sodium and alcohol: §

$$HO \cdot C_6H_4 \cdot CH_2 \cdot CN + 4H = HO \cdot C_6H_4 \cdot CH_2 \cdot CH_2 \cdot NH_2$$

2. From the benzoyl derivative of β-phenylethylamine by the following general reactions: ||

$$C_6H_5CH_2CH_2NHCOC_6H_5 \rightarrow NO_2 \cdot C_6H_4 \cdot CH_2 \cdot CH_2 \cdot NH \cdot COC_6H_5$$
$$\rightarrow NH_2 \cdot C_6H_4 \cdot CH_2 \cdot CH_2NHCOC_6H_5$$
$$\rightarrow HO \cdot C_6H_4 \cdot CH_2 \cdot CH_2NH \cdot CO \cdot C_6H_5 \rightarrow HO \cdot C_6H_4 \cdot CH_2 \cdot CH_2 \cdot NH_2$$

* Wohl and Berthold, *Ber.*, 1910, **43**, 2175.

† Barger and Walpole, *J. Physiol.*, 1909, **38**, 343.

‡ Barger and Dale, *ibid.*, 1909, **38**, 67. § Barger, *Trans.*, 1909, **95**, 1123.

|| Barger and Walpole, *Trans.*, 1909, **95**, 1720.

3. By reduction of the condensation product of anisaldehyde with nitromethane. The *p*-methoxyphenylethylamine thus obtained is then boiled with hydriodic acid.*

$$CH_3O \cdot C_6H_4 \cdot CHO + CH_3NO_2 \rightarrow CH_3O \cdot C_6H_4CH : CH \cdot NO_2$$
$$\rightarrow CH_3O \cdot C_6H_4 \cdot CH_2CH{:}NOH \rightarrow CH_3O \cdot C_6H_4 \cdot CH_2 \cdot CH_2NH_2$$
$$\rightarrow HO \cdot C_6H_4 \cdot CH_2 \cdot CH_2NH_2$$

This base produces physiological effects of the same type as those produced by adrenaline, although its activity is relatively small. In the body it is partly converted into *p*-hydroxyphenylacetic acid ($HO \cdot C_6H_4 \cdot CH_2 \cdot COOH$).†

A number of *p*-hydroxyphenylethylalkyl amines have been prepared by Walpole and their physiological activity has been studied by Dale.

Hordenine, $p\text{-}HO \cdot C_6H_4 \cdot CH_2 \cdot CH_2 \cdot N(CH_3)_2$, was obtained from an infusion of barley germs by Léger. The base has only a transitory existence during the germination of barley and has feeble pressor action. Three syntheses of this base may be briefly described.

1. Barger‡ obtained hordenine from β-phenylethylalcohol by the following general reactions:

$$C_6H_5CH_2 \cdot CH_2OH \rightarrow C_6H_5CH_2CH_2Cl \rightarrow C_6H_5 \cdot CH_2 \cdot CH_2 \cdot N(CH_3)_2$$
$$\rightarrow NO_2 \cdot C_6H_4 \cdot CH_2 \cdot CH_2N(CH_3)_2 \rightarrow NH_2 \cdot C_6H_4 \cdot CH_2 \cdot CH_2N(CH_3)_2$$
$$\rightarrow HO \cdot C_6H_4 \cdot CH_2 \cdot CH_2N(CH_3)_2$$

2. Rosenmund methylated *p*-methoxyphenylethylamine to the tertiary base hordenine methyl ether, from which hordenine was obtained by boiling with hydriodic acid: §

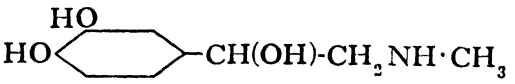
$$CH_3O \cdot C_6H_4 \cdot CH_2 \cdot CH_2 \cdot NH_2 \rightarrow CH_3O \cdot C_6H_4 \cdot CH_2 \cdot CH_2 \cdot N \cdot (CH_3)_2$$
$$\rightarrow HO \cdot C_6H_4 \cdot CH_2 \cdot CH_2 \cdot N \cdot (CH_3)_2$$

3. By distillation in vacuo of quaternary hordenine methiodide, obtained by complete methylation of *p*-hydroxyphenylethylamine: ||

$$HO \cdot C_6H_4 \cdot CH_2 \cdot CH_2 \cdot N(CH_3)I \rightarrow HO \cdot C_6H_4 \cdot CH_2 \cdot CH_2N(CH_3)_2 + CH_3I$$

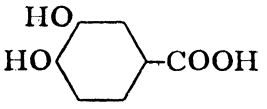
* Rosenmund, *Ber.*, 1909, **42**, 4778.
† Ewins and Laidlow, *J. Physiol.*, 1910, **41**, 78.
‡ *Trans.*, 1909, **95**, 2193. § *Ber.*, 1910, **43**, 306. || *D. R. P.*, 233069

Adrenaline (epinephin),

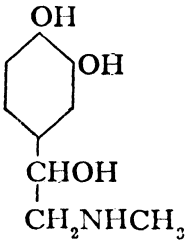


Although nothing is known of the nature of the parent substance from which adrenaline is derived, yet the base is obviously more closely related to tyrosine than to any other known constituent of protein. The physiological importance of the supra-renal glands was first made clear by Addison in 1849, and in 1894 Oliver and Schäfer observed the remarkable rise of blood pressure caused by the injection of supra-renal extracts. Takamine isolated the active principle of the glands in 1901, and Aldrich * assigned to it the correct empirical formula in the same year.

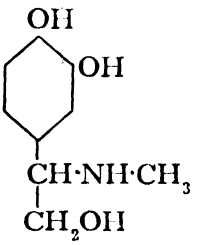
Natural adrenaline contains a methylamino group, and an alcoholic hydroxyl group, and on fusion with potash yields protocatechuic acid:



Pauly † showed that adrenaline contains an asymmetric carbon atom, and reduced the possible constitutional formulæ to two:



(i)



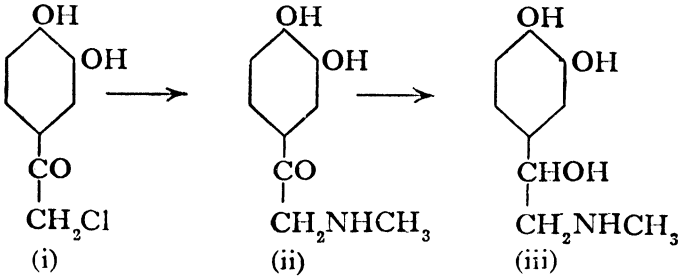
(ii)

Jowett ‡ arrived at similar results and favoured the first formula. The constitutional formula (i) of adrenaline was established by its synthesis by Stolz § and almost simultaneously by Dakin,‖ and the subsequent resolution of the synthetic product by Flacher,** the lævo form of which was completely identical with natural adrenaline.

Adrenaline has been synthesized by several methods, of which the first is the most important.

* *Amer. J. Physiol.*, 1901, **5**, 457.
† *Ber.*, 1903, **36**, 2944.
‡ *Trans.*, 1904, **85**, 192.
§ *Ber.*, 1904, **37**, 4149.
‖ *Proc. Roy. Soc.*, 1905, **76** [B], 491, 498.
** *Zeit. physiol. Chem.*, 1908, **58**, 581.

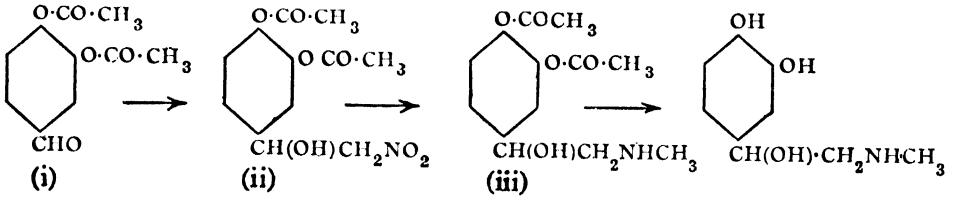
1. Catechol is condensed with monochloracetic acid in the presence of phosphorus oxychloride (or with chloracetyl chloride in the presence of aluminium chloride), and the resulting chloroacetocatechol (i) treated, in alcholic solution, at ordinary temperature, with a concentrated aqueous solution of methylamine. The methylaminoacetocatechol (ii) so obtained is then reduced to racemic adrenaline (iii) by means of aluminium amalgam, or electrolytically.*



2. Protocatechic aldehyde is converted into the cyanhydrin (i), which, on reduction, gives 3 : 4-dihydroxyphenylethanolamine (ii). This base is about as active as adrenaline, and is known commercially as " arterenol ".† On methylation it is said to yield adrenaline.

$$(HO)_2 \cdot C_6H_3 \cdot CH(OH)CN \longrightarrow (HO)_2 \cdot C_6H_3 \cdot CH(OH)CH_2NH_2.$$

3. According to the method of Nagai,‡ diacetylprotocatechic aldehyde (i) is condensed with nitromethane, and the product (ii) is mixed with the calculated quantity of formaldehyde and reduced by zinc dust and acetic acid to give β-hydroxy-β-3 : 4-diacetoxyphenylethylmethylamine (iii). On removing the acetyl groups from this compound, adrenaline is obtained:



Racemic adrenaline may be resolved into its optical isomers with the aid of *d*-tartaric acid. The *d*-adrenaline obtained as a by-product is then racemized by means of acids. Lævoadrenaline has many times the pressor effect of the dextro form.

Several investigators, and particularly Barger and Dale,§ have

* *D. R. P.*, 152814, 157300. † *D. R. P.*, 193634.
‡ *Japs. Pat.*, 1918, 32440, 32441.
§ *J. Physiol.*, 1910, **41**, 19; see also Tutin, *Trans.*, 1910, **97**, 2496.

prepared compounds analogous in chemical structure to adrenaline, and have examined their physiological action. The latter investigators have examined a large number of amines, and have shown that an action simulating that of adrenaline is not peculiar to this substance alone, but is possessed by a large series of amines, the simplest being primary aliphatic amines. The most active of the simpler bases is β-phenylethylamine. The presence of two hydroxyl groups in the 3:4 position of the nucleus increases the effect, which is further intensified by a hydroxyl group in the side chain. In short, the natural product seems to be the best adapted for this special function.

DIAMINO BASES

Putrescine (tetramethylene diamine, $NH_2[CH_2]_4NH_2$) and **Cadaverine** (pentamethylene diamine, $NH_2[CH_2]_5NH_2$) are of historic interest, as they were among the earliest putrefaction bases to be isolated and characterized. They are, however, comparatively innocuous substances, having very slight physiological activity. Apart from the bacterial formation of putrescine and cadaverine, both bases have been isolated from ergot. Putrescine further occurs in Thorn-apple (*Datura*), and tetramethyl putrescine in a species of Henbane (*Hyoscyamus muticus*).

Both bases were prepared by Ladenburg in 1886, by reducing the necessary cyanides with sodium in hot alcohol.

$$Br\cdot[CH_2]_x Br \rightarrow CN\cdot[CH_2]_x CN \rightarrow NH_2\cdot CH_2[CH_2]_x CH_2NH_2$$

The origin of both amines was definitely established by Ellinger,* who obtained putrescine by the action of putrefactive bacteria on ornithine and similarly cadaverine from lysine:

$NH_2\cdot[CH_2]_3CH(NH_2)COOH$	$NH_2[CH_2]_4\cdot NH_2$
Ornithine	Putrescine
$NH_2[CH_2]_4CH(NH_2)COOH$	$NH_2[CH_2]_5NH_2$
Lysine	Cadaverine

Agmatine, guanidinobutylamine, has been isolated from ergot, and also obtained by heating herring spawn with dilute acid under pressure. On oxidation it yields guanidine and guanidino-

* *Zeit. physiol. Chem.*, 1900, **29**, 334.

butyric acid, and it is probably derived from arginine by decarboxylation:

$NH_2C(:NH) \cdot NH \cdot [CH_2]_4 \cdot NH_2$ Agmatine

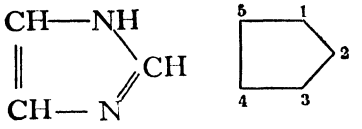
$NH_2C(:NH) \cdot NH \cdot [CH_2]_3CH(NH_2)COOH$ Arginine

Kossel* has synthesized agmatine from cyanamide and tetramethylene diamine:

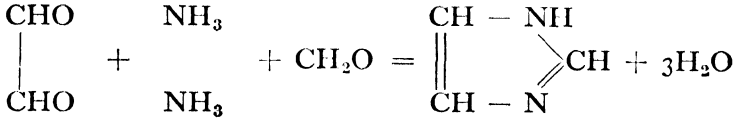
$$NH_2CN + NH_2[CH_2]_4NH_2 = NH_2 \cdot C[:NH] \cdot NH \cdot [CH_2]_4NH_2$$

HETEROCYCLIC BASES

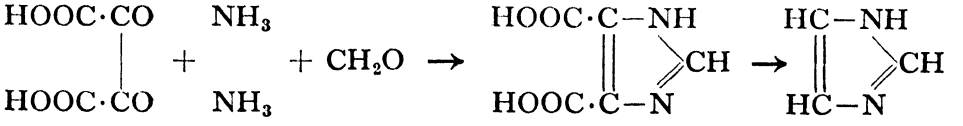
Glyoxaline, imidazole,



This compound may be regarded as the parent of several of the heterocyclic bases about to be described, and although it has not been directly obtained as a product of plant or animal metabolism, a short description of this compound appears to be advisable. Glyoxaline was obtained by Debus † as early as 1858 by the action of ammonia on glyoxal. During this reaction a portion of the glyoxal is converted into formic acid and formaldehyde, and the latter combines with ammonia and unchanged glyoxal to give glyoxaline:



Glyoxaline may best be obtained by the action of ammonia on a mixture of formaldehyde and dinitrotartaric acid,‡ followed by elimination of carbon dioxide from the resulting glyoxaline dicarboxylic acid at 300°. In this reaction diketosuccinic acid is presumably formed:

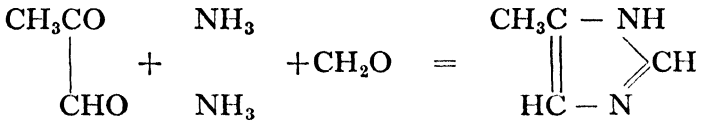


* *Zeit. physiol. Chem.*, 1910, **68**, 170. † *Ann.*, **107**, 204.

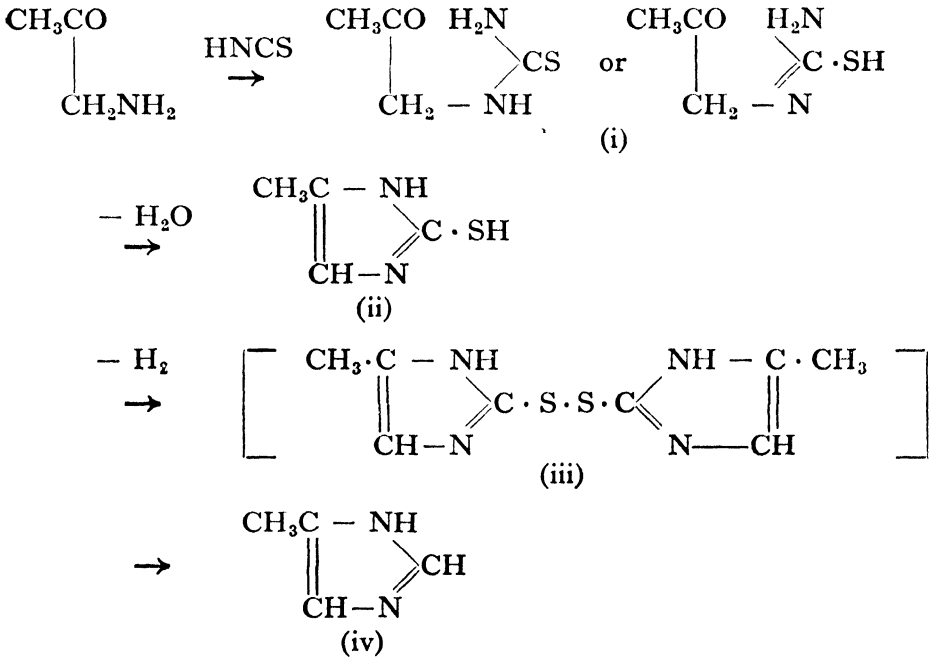
‡ Maquenne, *Ann. Chim.*, 1891, **24**, 528; Fargher and Pyman, *Trans.*, 1919, **115**, 217.

Glyoxaline forms thick, colourless prisms, and an aqueous solution reacts alkaline. The silver salt is insoluble in water. Ordinary reducing agents have no action on the base. With hydrogen peroxide, glyoxaline is oxidized to oxamide, while potassium permanganate gives formic acid. The glyoxaline nucleus has been the object of special study by Pyman and Fargher.*

It is interesting to note that Windaus and Knoop † have obtained 4 (or 5)-methylglyoxaline by the action of zinc ammonium hydroxide on glucose and other monosaccharoses. In this reaction it is assumed that methylglyoxal and formaldehyde are formed as intermediate products:



The formation of glyoxaline derivatives from the α-amino derivatives of aldehydes, acetals, or ketones with the aid of the thiocyanates is a reaction of considerable importance. This reaction was first discovered by Wohl and Marckwald,‡ and may be illustrated by the synthesis of 4 (or 5)-methylglyoxaline.

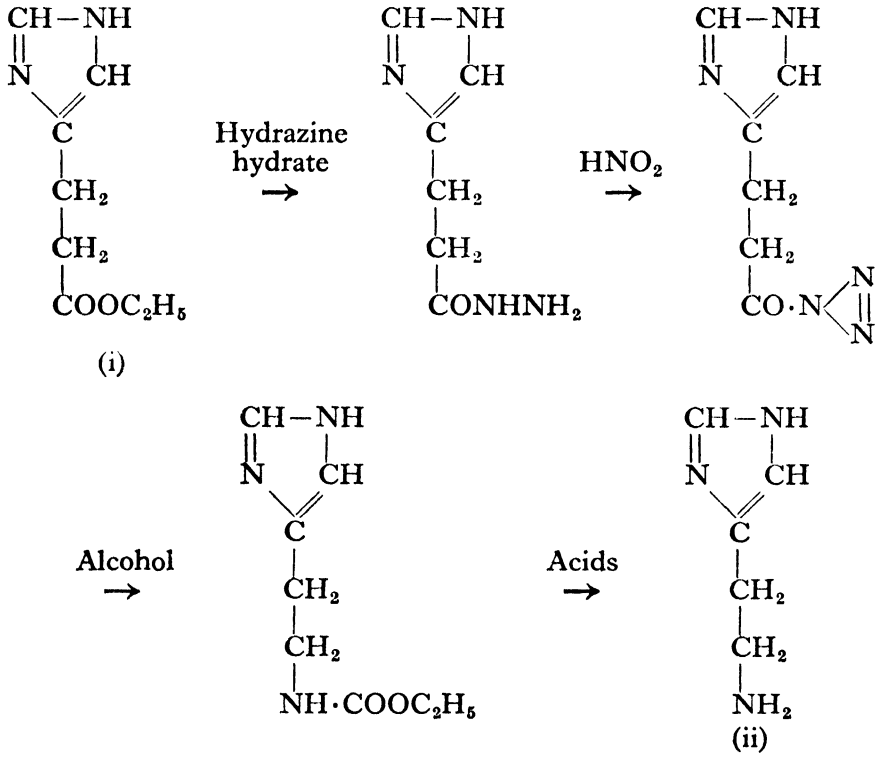


* *Trans.*, 1919, **115**, 217, 1016; Fargher, *T.*, 1920, **117**, 668; 1921, **119**, 158.
† *Ber.*, 1905, 38, 1166. ‡ *Ber.*, 1889, **22**, 572, 1353.

Aminoacetone is condensed with potassium thiocyanate to give the substituted thiourea (i). On warming with hydrochloric or sulphuric acid, mercaptoglyoxaline (ii) is obtained. On treatment with warm dilute nitric acid 4 (or 5)-methylglyoxaline (iv) is formed, possibly through the intermediate disulphide (iii). This reaction has been used extensively in the synthesis of glyoxaline derivatives. N-alkyl- or aryl-glyoxalines are obtained when the alkyl- or aryl-isothiocyanates are used instead of potassium thiocyanate.

Histamine, 4-β-aminoethylglyoxaline, β-iminazoylethylamine. —In 1910 Ackermann * obtained a large yield of this base by the putrefaction of histidine (p. 160). A little later Barger and Dale,† and simultaneously Kutscher,‡ obtained the same base from ergot. The physiological activity of this base is very pronounced, and in minute doses it produces chronic contraction of the uterus.

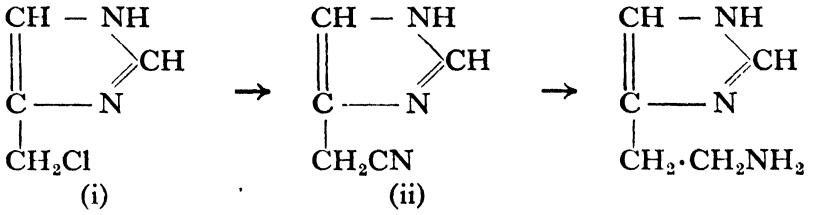
Histamine was first obtained synthetically by Windaus and Vogt. § For this purpose glyoxaline-4-propionic ester (i) was converted into 4-β-aminoethylglyoxaline (ii) by Curtius' method:



(i) (ii)

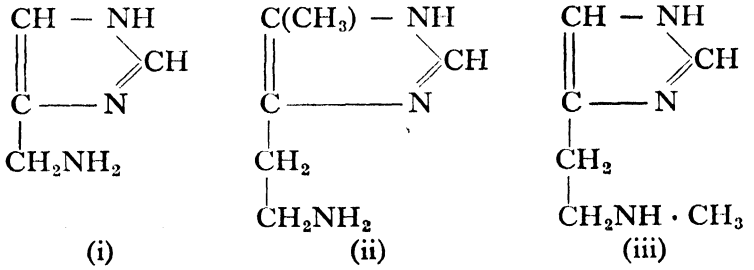
* *Zeit. physiol. Chem.*, 1910, 65, 504.
† *Trans.*, 1910, **97**, 2592.
‡ *Zeit. Physiol.*, 1910, **24**, 163.
§ *Ber.*, 1907, **40**, 3691.

This method is tedious and expensive, and a more satisfactory method was devised by Pyman.* For this purpose 4 (or 5)-chloromethylglyoxaline (i) is converted into the corresponding cyanide and reduced by sodium and alcohol to histamine.



(i) (ii)

4-β-aminomethylglyoxaline (i), the lower homologue of histamine, was synthesized from glyoxaline-4-acetic acid by a similar method to that which Windaus and Vogt employed for the synthesis of histamine. It may also be obtained from diamino-acetone by means of the mercaptan reaction already described (p. 161),† but the base is almost devoid of physiological action. Ewins ‡ synthesized 4-methyl-β-aminoethyglyoxaline (ii), and found it to be somewhat less powerful than 4-β-aminoethylglyoxaline. Still more recently Fargher and Pyman § have prepared 4-β-methylaminoethylglyoxaline (iii), but its physiological action is weak.



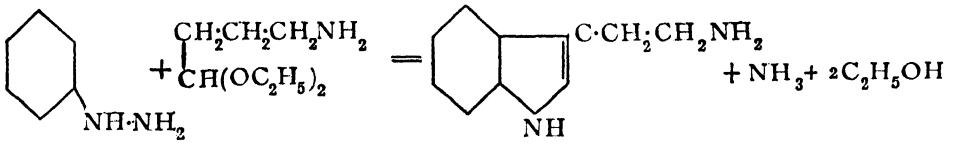
(i) (ii) (iii)

BASES DERIVED FROM TRYPTOPHANE

Indolethylamine (3-β-aminoethylindole).—Tryptophane, unlike tyrosine, cannot be decarboxylated by heat. Indolethylamine was obtained by Ewins and Laidlow || both synthetically and by the action of putrefactive bacteria on tryptophane. The synthesis, subsequently described by Ewins ** was carried out along the lines of the well-known phenylhydrazone method for the synthesis of indole derivatives.†† The requisite aldehyde could not be isolated

* *Trans.*, 1911, 99, 668. † *Ber.*, 1911, 44, 1721. ‡ *Trans.*, 1911, 99, 2052.
§ *Trans.*, 1921, 119, 734. || *Proc.*, 1910, 27, 343. ** *Trans.*, 1911, 99, 270.
†† Fischer, *Ann.*, 1886, 236, 137.

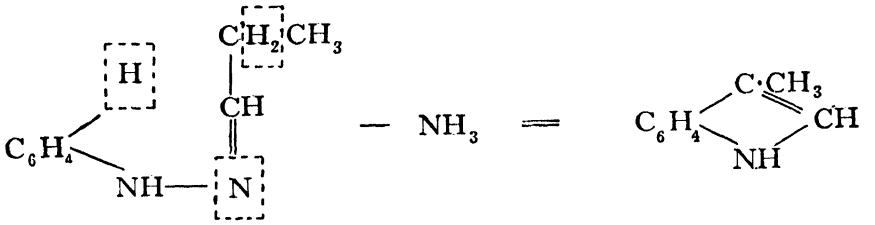
in the free state, so the corresponding acetal was employed. Phenylhydrazine was condensed with γ-aminobutyrylacetal in the presence of zinc chloride, and the base isolated as its picrate:



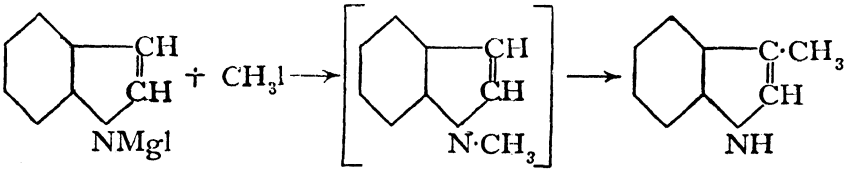
Indolethylamine produces a transient stimulant effect upon the central nervous system, and acts as a direct stimulant on plain muscle.*

Scatole (β-methylindole).—Scatole was isolated from human fæces by Brieger in 1877. It represents a further stage of putrefactive decomposition in which decarboxylation and deamination are succeeded by partial oxidation of the side chain of tryptophane. Scatole has been isolated by Dunstan † and by Herter ‡ from the wood of *Celtis reticulosa*, which grows in Java and Ceylon.

Methylindole is readily obtained by the action of zinc chloride on the phenylhydrazone of propionaldehyde:§



or by the action of methyliodide on magnesium indolyliodide:‖

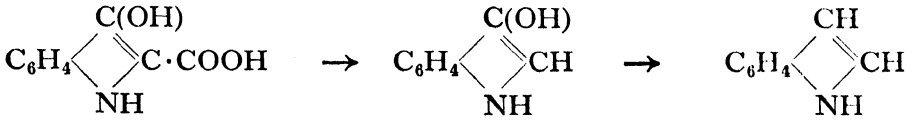


Magnesium indolyliodide is readily obtained by the action of magnesium alkyliodides on indole.

Indole.—In the formation of indole complete oxidation of the side chain of the tryptophane molecule has occurred.

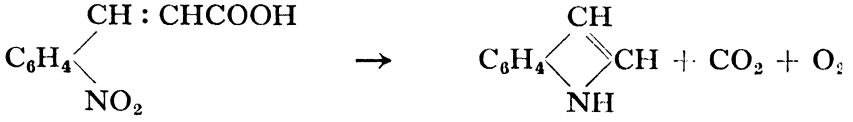
* Laidlow, *Biochem. J.*, 1911, 6, 141. † *Proc. Roy. Soc.*, 1889, 46, 211.
‡ *J. Biol. Chem.*, 1909, 5, 489. § Fischer (*loc. cit.*).
‖ Oddo, *Gazz.*, 1911, 41, i, 229.

Indole was first obtained by Baeyer by distilling with zinc dust, either oxindole, $C_6H_4\langle\begin{matrix}CH_2\\NH\end{matrix}\rangle CO$, or the product obtained by reducing indigo with tin and hydrochloric acid. It is conveniently prepared by the reduction of indoxyl, obtained by heating indoxylic acid with sodium-amalgam or zinc dust.*



Indoxylic acid — Indoxyl — Indole

Baeyer and Emmerling † obtained indole by distilling *o*-nitrocinnamic acid with caustic potash and iron filings:

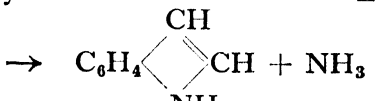


o-Nitrocinnamic acid — Indole.

Indole is said to be obtained when the condensation product of dichlorethylether and aniline is distilled in steam.

$$CH_2Cl\cdot CHCl\cdot O\cdot CH_2CH_3 + 2C_6H_5NH_2 \rightarrow C_6H_5N{:}CH\cdot CH_2\cdot NH\cdot C_6H_5$$

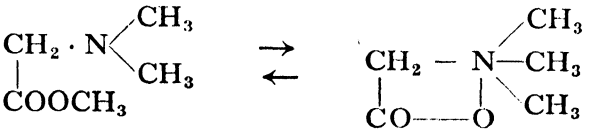
Dichlorethylether — Ethylidene dianiline



Indole

THE BETAÏNES

The betaïnes are amino acids in which the nitrogen atom is directly attached to two methyl groups. They may be classified as α, β, or γ compounds according as they are derived from α, β, or γ amino acids. Willstätter ‡ has made a detailed study of the betaïnes, and has shown that the α betaïnes and the isomeric esters of dimethylamino acids are interconvertible:



This change only proceeds from left to right in the case of the betaïnes of β and γ amino acids. The α betaïnes differ considerably

* *Ber.*, 1904, 37, 1134. † *Ber.*, 1869, 2, 680. ‡ *Ber.*, 1902, 35, 584.

in stability and are so unstable that they cannot be formed by the ordinary process of methylation; e.g. aspartic acid, when treated with methyliodide and alkali, breaks up into trimethylamine and fumaric acid.

When the betaïnes are dried above 100° their composition corresponds to the cyclic anhydride structure. Many of the betaïnes crystallize with one molecule of water, and in this condition their constitution is best expressed by the open-chain formula.

Betaïne, trimethyglycine, $\begin{matrix} CH_2 - N(CH_3)_3 \\ | \quad\quad | \\ CO\text{——}O \end{matrix}$, was first isolated from *Lycium barbarum* in 1863. It has been found in all species of Chenopodiaceæ so far examined, including the sugar beet (*Beta vulgaris*), from which the compound derives its name. In the manufacture of beet sugar most of the betaïne remains in the molasses, and after desaccharification the final liquor, called " Schlempe ", is very rich in betaïne.

Betaïne was first obtained synthetically by Liebrich by the action of monochloracetic acid on trimethylamine.

$$(CH_3)_3N + CH_2ClCOOH \rightarrow (CH_3)_3N\begin{matrix} Cl \\ \diagup \\ \diagdown \\ CH_2COOH \end{matrix} \rightarrow (CH_3)_3N\begin{matrix} O \\ \diagup\diagdown CO \\ CH_2 \end{matrix}$$

The same product was obtained by the methylation of glycine by Griess in 1875.

CHOLINE AND ALLIED BASES

In a combined form, choline is probably present in every living cell. Choline enters into the composition of the phosphatides (p. 104), and it may be considered as the fundamental unit or " Bausteine " of the phosphatides.

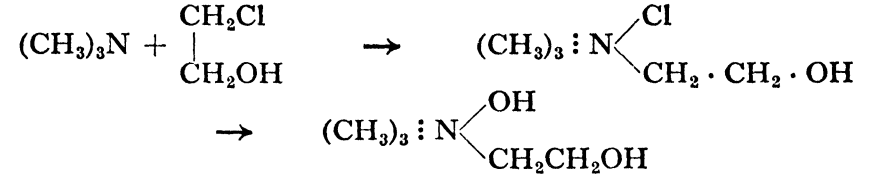
Choline (trimethyl - β - hydroxyethyl - ammonium hydroxide)

$$(CH_3)_3 : N\begin{matrix} OH \\ \diagup \\ \diagdown \\ CH_2 \cdot CH_2OH \end{matrix}$$

was discovered by Strecker in 1849. It is most readily obtained by hydrolyzing lecithin with baryta and subsequently precipitating the base with alcoholic platinic chloride. The synthesis of choline has been effected in a variety of ways, among which may be mentioned:

1. The action of trimethylamine on ethylene oxide in aqueous solution (Wurtz, 1867):

2. By the action of trimethylamine on ethylene chlorhydrin, and subsequent decomposition of the chloride of the base with silver oxide:*

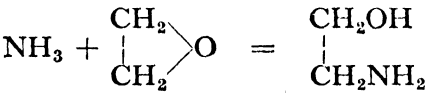


According to Ewins,† acetyl choline

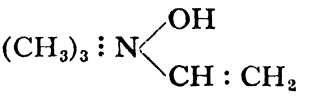
$$[(CH_3)_3N(OH) \cdot CH_2 \cdot CH_2 \cdot O \cdot CO \cdot CH_3]$$

is present in small quantity in some ergot extracts. The physiological action of a number of esters and ethers of choline has been studied by Dale.

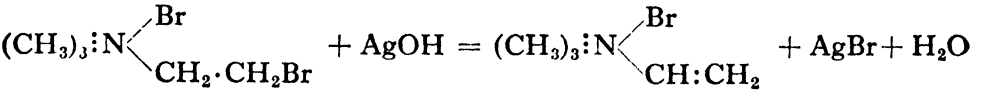
Amino-ethyl Alcohol is prepared from kephalin (a phosphatide from the brain) in a similar manner to that by which choline is obtained from lecithin. It has been synthesized by Knorr by the action of ammonia on ethylene oxide:



Neurine (vinyltrimethyl-ammonium hydroxide)



occurs as a product of putrefaction and was isolated by Brieger in 1885 from putrid meat. Its structure is determined by its relation to choline, and by its synthesis from trimethylamine and ethylene bromide. The condensation product obtained from these substances yields neurine on treatment with moist silver oxide (Hofmann, 1858):

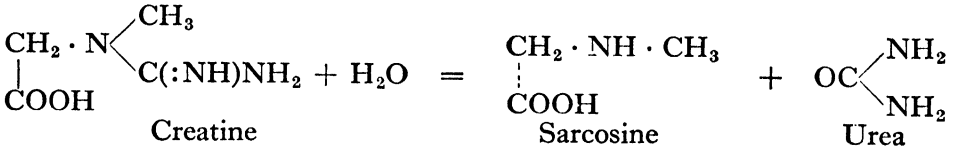


Neurine is a powerfully toxic compound, and in its physiological action resembles choline.

* Renshaw, *J. Amer. Chem. Soc.*, 1910, 32, 128. † *Biochem. J.*, 1914, 8, 44.

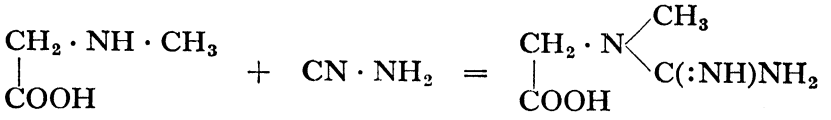
CREATINE AND SOME ALLIED SUBSTANCES

Creatine was first described by Chevreul in 1835, and was studied by Liebig in his classical investigation of the constituents of muscle juice. It is a constituent of all vertebrate muscle, and is found in the juice of flesh to the extent of about 6 per cent. On hydrolysis with baryta it is converted into sarcosine (methylglycine) and urea:

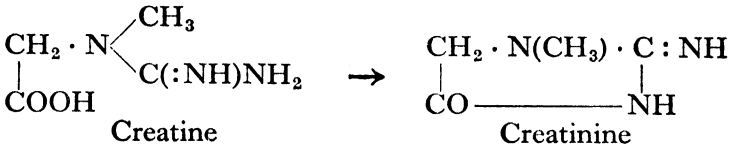


Creatine — Sarcosine — Urea

In 1868 Volhard synthesized creatine by the action of cyanamide on sarcosine in alcoholic solution at 100°:



Creatinine is absent from muscle but is a normal constituent of the urine of mammals. It may be obtained from creatine by the action of heat or dehydrating agents:



Creatine — Creatinine

The reaction may be reversed by the action of alkalies. Both creatine and creatinine occur in cereals.

Guanidine, $HN:C(NH_2)_2$, has been isolated from Vicia seedlings by Schultze. It is most conveniently prepared by heating ammonium thiocyanate:

$$2NH_4SCN \rightarrow \underset{\text{Thiourea}}{2CS(NH_2)_2} \rightarrow \underset{\text{Guanidine thiocyanate}}{NH:C(NH_2)_2, HCNS} + H_2S$$

More recently guanidine thiocyanate has been obtained by Werner,* in a 90 per cent yield, by heating a mixture of dicyanodiamide and ammonium thiocyanate at 120°. The first phase of this reaction is probably the depolymerization of dicyanodiamide, the guanidine salt being formed by the union of cyanamide and ammonium thiocyanate thus:

$$CN \cdot NH_2 + NH_3 \cdot HSCN = HN:C(NH_2)HSCN$$

* *Trans.*, 1920, **117**, 1133.

Guanidine is a very soluble, deliquescent, crystalline base which absorbs carbon dioxide freely, forming a carbonate.

Methylguanidine, $H_2N \cdot C(=NH)(NHCH_3)$, is a normal constituent of muscle. It may be obtained by the oxidation of creatine, or synthetically from cyanamide and methylamine:

$$NH_2 \cdot CN + NH_2CH_3 = HN : C(NH_2)(NHCH_3)$$

Methylguanidine

Werner and Bell * have prepared methylguanidine hydrochloride by heating a mixture of dicyanodiamide and methylammonium chloride for three hours at 180°.

THE ω-AMINO ACIDS

The monoamino acids which have been described in the previous chapter contain the amino group in the α position, and the basic amino group is more or less neutralized by the presence of a carboxyl group attached to the same carbon atom, so that only the diamino acids are basic. When the amino group is not in the α position the basic character is more pronounced, and the so called ω-amino acids are weak bases. These amino acids can be precipitated by phosphotungstic acid. The γ, δ, and ε amino acids are so weakly acidic that they do not form copper salts.

The occurrence and synthesis of a few of these substances may be briefly considered.

β-Alanine, β-aminopropionic acid, $NH_2 \cdot CH_2 \cdot CH_2 \cdot COOH$.—This substance was first obtained synthetically by Heintz in 1870 by the action of ammonia on β-iodopropionic acid. It may also be obtained by the reduction of cyanacetic acid with zinc and sulphuric acid.

It is best prepared synthetically by the action of bromine and alkali on succinimide (Hofmann's reaction):

$$CH_2 - CO \\ | \quad \quad >N \cdot Br \rightarrow CH_2 \cdot C(:NBr)OK \\ CH_2 - CO \quad \quad | \\ \quad \quad \quad \quad CH_2 \cdot COOK$$

$$\rightarrow CH_2 \cdot N : CBr(OK) \rightarrow CH_2 \cdot NH_2 \\ \quad \quad | \quad \quad \quad \quad | \\ \quad \quad CH_2 \cdot COOK \quad \quad CH_2 \cdot COOK$$

* *Trans.*, 1922, **122**, 1793.

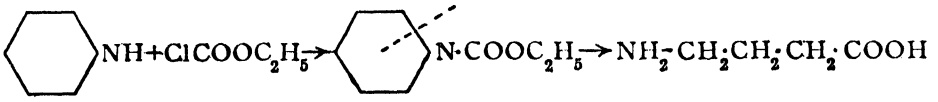
Alanine was first isolated from Liebig's extract of meat by Engeland. Since it is obtained from the meat base carnosine by hydrolysis, it is doubtful if β-alanine is present as such in muscle.

γ-Amino-*n*-butyric Acid, $NH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH$.—This acid is produced in putrefaction by the partial decarboxylation of glutamic acid:

$$COOH \cdot CH(NH_2) \cdot CH_2 \cdot CH_2 \cdot COOH = NH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2COOH + CO_2$$

γ-Amino-*n*-butyric acid has been synthesized from γ-chlorobutyronitrile, $ClCH_2CH_2 \cdot CH_2CN$, by means of the phthalimide reaction (p. 147).

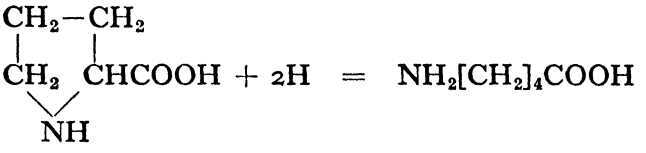
It may also be obtained by the oxidation of piperidine urethane by nitric acid:



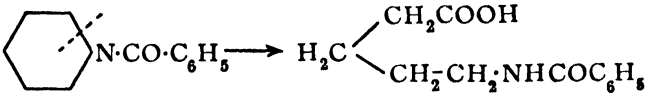
δ-Amino-*n*-valeric Acid, $NH_2 \cdot (CH_2)_4 \cdot COOH$.—This acid was obtained by E. and H. Salkowski in 1883 from putrefied muscle and fibrin. During putrefaction it may be obtained by partial deaminization of ornithine:*

$$NH_2 \cdot [CH_2]_3CH(NH_2)COOH + 2H = NH_2[CH_2]_3CH_2COOH + NH_3$$

or by the reduction of proline (p. 157):



It was first obtained synthetically by hydrolysis of the oxidation product of benzoyl piperidine with potassium permanganate: †

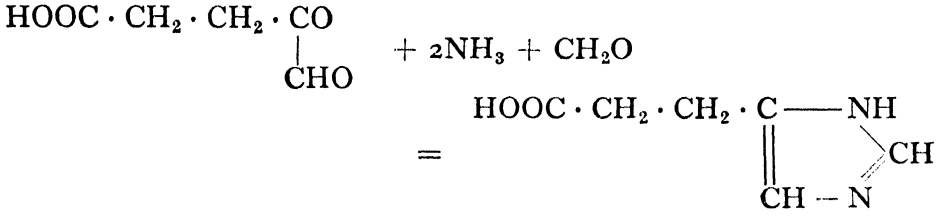


It may also be obtained synthetically by a combination of the phthalimide and malonic ester reactions.‡

β-Iminazoylpropionic Acid.—By the putrefaction of histi-

* Ackermann, *Zeit. Biol.*, 1911, **57**, 104; Neuberg, *Biochem. Zeit.*, 1911, **37**, 490.
† Schotten, *Ber.*, 1884, **17**, 2544; 1888, **21**, 2240.
‡ Gabriel, *Ber.*, 1890, **23**, 1767; 1891, **24**, 1364.

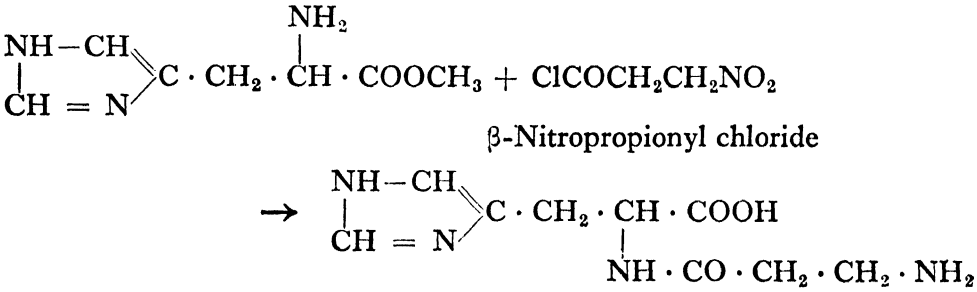
dine hydrochloride a small quantity of this acid, together with much iminazoylethylamine, is obtained. The acid may be obtained synthetically by the action of ammonia and formaldehyde on β-glyoxylpropionic acid:



β-Glyoxylpropionic acid is obtained by boiling dibromlevulinic acid with water.

Carnosine.—This is probably the base in muscle which gives rise to β-alanine on hydrolysis. Next to creatine it is the most abundant base in meat extract.

The constitution of carnosine as β-alanylhistidine has been established by its synthesis from histidine methyl ester by Barger and Tutin:*



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The Simpler Natural Bases, by G. Barger: Monographs on Biochemistry (London, 1914).

Biochemisches Handlexikon, Band 5, by Abderhalden (Berlin, 1911).

* *Biochem. J.*, 1918, **12**, 402.

CHAPTER IX

The Pyrimidine and Purine Bases

Introduction.—The alchemists were familiar with the peculiar concretionary conglomerates of urinary deposits known as urinary calculi, and it was from this source that Bergmann obtained uric acid in 1776. Almost contemporaneously, Scheele obtained the same acid, which he termed " lithic acid ", from human urine.

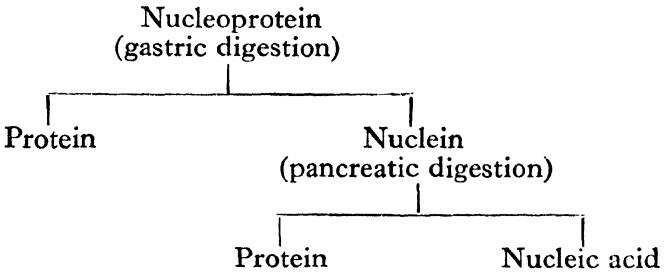
In 1838 Liebig and Wöhler published the results of their investigation of uric acid in which they showed that it yields a large number of compounds which may be grouped together as derivatives of alloxan and parabanic acid. In view of the state of chemical theory at the time, these investigations must be regarded as classical.

The study of these compounds was continued by Baeyer, and his results, published in 1863 and 1864, not only embraced the synthesis of pseudo-uric acid, but also prepared the way for the subsequent discovery of the structure and synthesis of uric acid and the allied xanthine or purine bases. Emil Fischer published the results of his investigation of caffeine in 1882, and this work ultimately led to the synthesis of uric acid, several xanthine bases, and purine—the parent substance of these compounds.

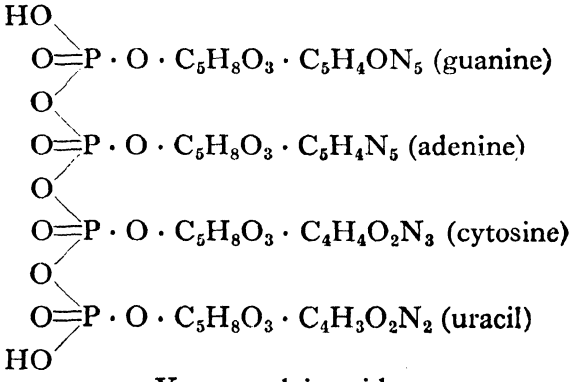
With the modern development of biochemistry the pyrimidine and purine derivatives have acquired a new significance, and the relation of the bases to these nucleic acids and nucleoproteins may be briefly considered.

The Nucleoproteins and Nucleic Acids.—The nucleoproteins are widely distributed in the animal and plant kingdoms, and are found in especially large amounts in glandular tissues such as those of the thymus, pancreas, and spleen. The nucleoproteins are combinations of proteins with phosphorus-containing substances known as nucleic acids. Under the action of the gastric juice or of weak acids nucleoproteins lose a portion of their protein content, and are transformed into a rather ill-defined class of substances known as nucleins which still possess some protein in combination

with the nucleic acid molecule. Through the action of pancreatic juice or further acid hydrolysis the remainder of the protein is split off and the nucleic acid set free.



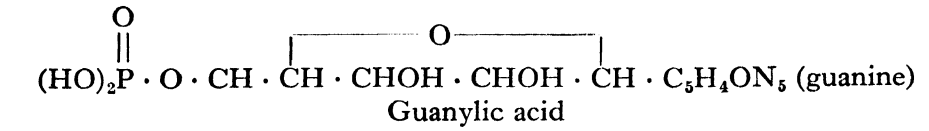
The nucleic acids probably comprise but two substances—animal nucleic acid and plant nucleic acid. Animal nucleic acid is most readily prepared from the thymus while plant nucleic acid is conveniently obtained from yeast. Nucleic acids readily undergo further hydrolysis by means of enzymes ("nucleases") present in most animal tissues. On complete hydrolysis the nucleic acids yield phosphoric acid, purine and pyrimidine bases, and a carbohydrate or carbohydrate derivative. The nucleic acids are not, however, simple substances whose molecules contain a single phosphoric acid, purine or pyrimidine group, and carbohydrate, but are apparently combinations of several radicles each of which contains these three compounds. Yeast nucleic acid is regarded as a combination of four nucleotides in which two pyrimidine and two xanthine bases are present:



Yeast nucleic acid

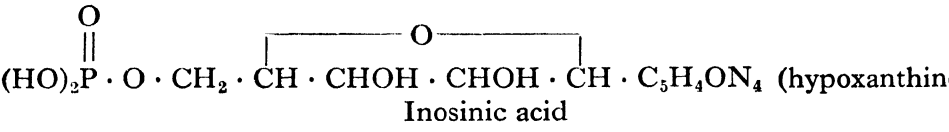
A simpler form of nucleic acid yields phosphoric acid, ribose, and guanine on hydrolysis, and has been termed guanylic acid. Inosinic acid is a similar compound which gives phosphoric acid, ribose, and hypoxanthine on hydrolysis. Levenne and Jacobs regard these

substances as mononucleotides and represent them by the formulæ:



$(HO)_2P \cdot O \cdot CH \cdot CH \cdot CHOH \cdot CHOH \cdot CH \cdot C_5H_4ON_5$ (guanine)

Guanylic acid



$(HO)_2P \cdot O \cdot CH_2 \cdot CH \cdot CHOH \cdot CHOH \cdot CH \cdot C_5H_4ON_4$ (hypoxanthine)

Inosinic acid

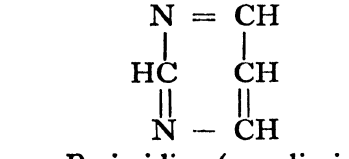
Under certain conditions partial hydrolysis may take place, in which case phosphoric acid is split off, and the compound of sugar and base which remains is called a nucleoside. For example, guanosin is guanine-*d*-riboside.

Plant nucleic acid contains a pentose group, while animal nucleic acid contains a hexose. Both types contain the purine bases, adenine and guanine, and the pyrimidine base cytosine. Uracil and thymine occur in plant and animal nucleic acid respectively. Nucleic acids from a variety of sources have been studied, but in no case have hydrolysis products other than those obtained by Kossel and his pupils from thymus and yeast nucleic acids been observed. These hydrolysis products may be tabulated:

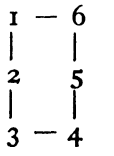
HYDROLYSIS PRODUCTS OF NUCLEIC ACIDS

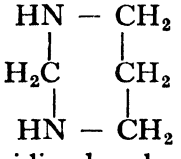
Of Animal Origin.		Of Plant Origin.
Lævulinic acid.	....	A Pentose (*d*-ribose).
Phosphoric acid.	....	Phosphoric acid.
Guanine.	....	Guanine.
Adenine.	....	Adenine.
Cytosine or Uracil.	....	Cytosine or Uracil.
Thymine.	....	Thymine.

PYRIMIDINE COMPOUNDS

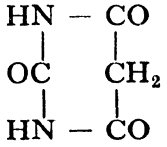


Pyrimidine (1:3-diazine)



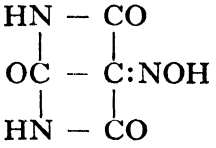


Pyrimidine hexahydride



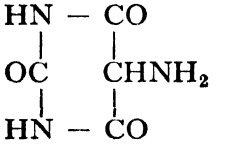
Barbituric acid

(2:4:6-Trioxopyrimidine hexahydride)



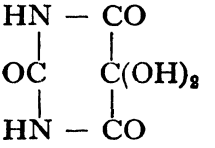
Violuric acid

(5-Oximino-2:4:6-trioxopyrimidine hexahydride)



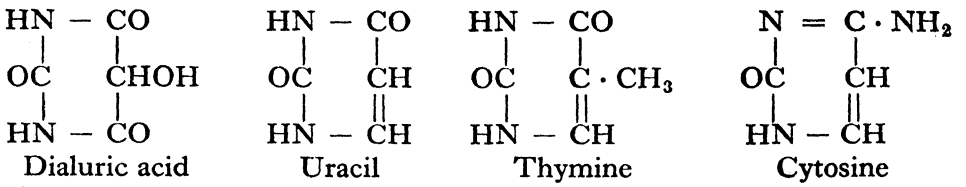
Uramil

(5-Amino-2:4:6-trioxopyrimidine hexahydride)



Alloxan

(5-Dihydroxy-2:4:6-trioxopyrimidine hexahydride)

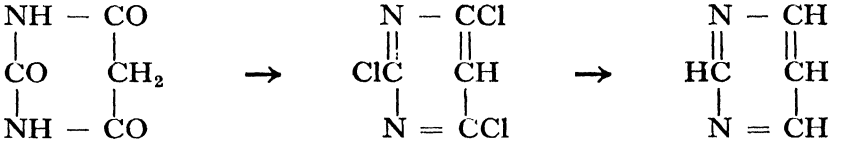


PYRIMIDINE AND SOME OF ITS DERIVATIVES

Before discussing the naturally occurring pyrimidine derivatives, pyrimidine itself and a few of its simpler derivatives may be considered on account of their relationship to the purine compounds.

Pyrimidine (1:3-diazine), $C_4H_4N_2$, the parent substance of the pyrimidine bases, has not been found among the decomposition products of the nucleic acids. The constitutional formula of this base is shown in the tabulation on p. 192, as well as that of a number of bases which may be considered as derivatives of pyrimidine or its fully saturated hexahydride.

Pyrimidine is most conveniently prepared from barbituric acid as follows.* Barbituric acid is converted into 2:4:6-trichloropurine on treatment with phosphorus oxychloride, and on reduction of this compound with zinc dust and hot water, pyrimidine is obtained:

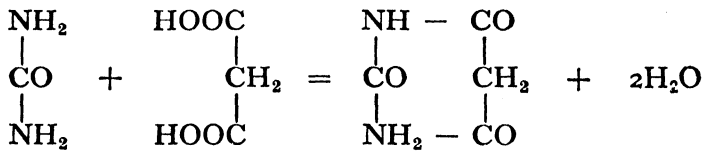


Pyrimidine is a colourless oil which slowly crystallizes at zero to a mass of fibrous crystals, melting-point 20° to 22°. Its aqueous solution is neutral to litmus, and gives a white precipitate with mercuric chloride ($C_4H_4N_2 + HgCl_2$), a yellow compound with gold chloride ($C_4H_4N_2 + AuCl_3$), and a picrate with picric acid ($C_4H_4N_2 + C_6H_3O_7N_3$).

Barbituric Acid (malonyl urea), $C_4H_4N_2O_3$, was first obtained by Baeyer during the course of his researches on the constitution of uric acid. It may be synthesized by the condensation of

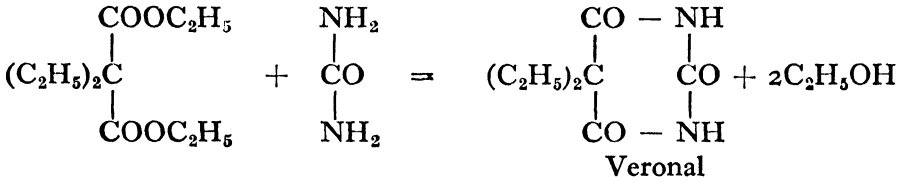
* Gabriel, *Ber.*, 1900, 33, 3666; Emery, *Ber.*, 1901, 34, 4180.

malonic acid and urea in the presence of phosphorus oxychloride: *

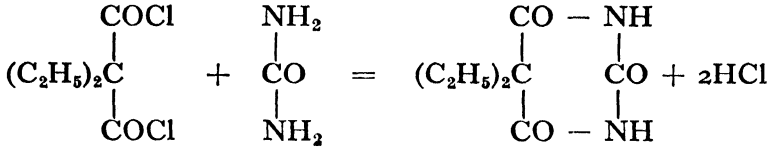


Barbituric acid

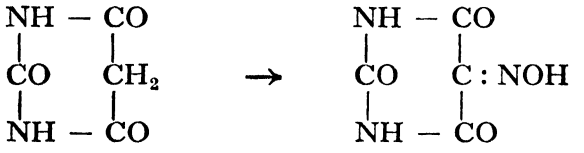
It is interesting to note that **Veronal**, one of the most valuable synthetic hypnotics, is closely related to barbituric acid, and may be prepared by combining diethylmalonic ester with urea in the presence of sodium ethoxide, or by the action of urea on diethylmalonyl chloride:



Veronal



Violuric Acid (isonitrosobarbituric acid), $C_4H_3N_3O_4$, may be conveniently obtained by the action of hydroxylamine on alloxan (p. 195), and also by the action of nitrous acid on barbituric acid. In the latter case the nitrous acid attacks the methylene group of the barbituric acid molecule:

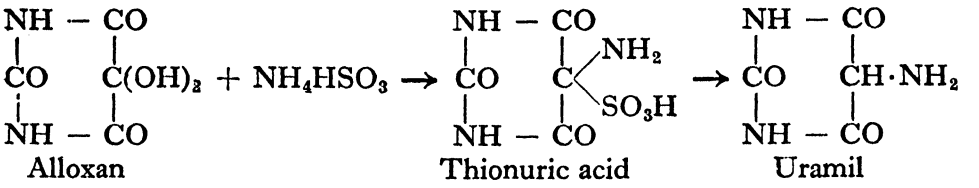


Barbituric acid — Violuric acid

Violuric acid and its salts were extensively studied by Hantzsch during the course of his researches on chromoisomerism.

Uramil (amidomalonyl urea), $C_4H_5N_3O_3$, may be obtained by the reduction of violuric acid or by boiling thionuric acid with water. The latter was prepared from alloxan by Liebig and Wöhler by the action of ammonium sulphite and an excess of ammonium carbonate:

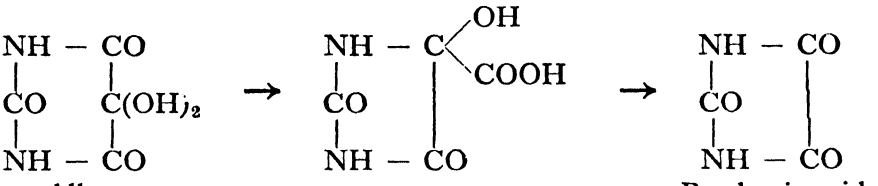
* Grimaux, *Bull. Soc. chim.*, 1876, 31, 146.



Alloxan — Thionuric acid — Uramil

Alloxan (mesoxalyl urea), $C_4H_4O_5N_2$, is readily obtained by the action of strong nitric acid on uric acid, and it was obtained in this manner as early as 1817 by Brugnatelli. Its constitutional formula was determined by Baeyer, and it is one of the few organic compounds in which two hydroxyl groups are attached to the same carbon atom.

By the action of strong reducing agents alloxan is converted into **dialuric acid*** (tartronyl ureide), while energetic oxidizing agents transform alloxan into **parabanic acid**,† probably according to the following scheme:



Alloxan — Parabanic acid

Uracil (2 : 6-dioxypyrimidine tetrahydride) **and Thymine** (5-methyluracil). — The term uracil was applied by Behrend to a hypothetical substance, the derivatives of which he had obtained during the course of his researches on uric acid. Uracil itself was first obtained in 1900 by Ascoli ‡ as a product of hydrolysis of yeast nucleic acid. Since that time it has frequently been encountered as a hydrolytic product of nucleic acid from various sources, especially when the latter is hydrolyzed by acids under pressure. Under these circumstances it is probably formed by the hydrolysis of cytosine:

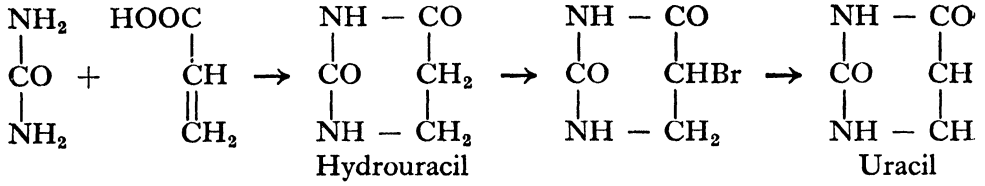
$$\underset{\text{Cytosine}}{C_4H_3N_2O(NH_2)} + H_2O = \underset{\text{Uracil}}{C_4H_3N_2O(OH)} + NH_3$$

Thymine was first obtained by the hydrolysis of the nucleic acid from the thymus gland by Kossel and Neumann in 1893, and was soon afterwards obtained from nucleic acid from other sources.

Fischer and Roeder§ obtained hydrouracil by the condensation of acrylic acid and urea, and by the action of bromine in glacial

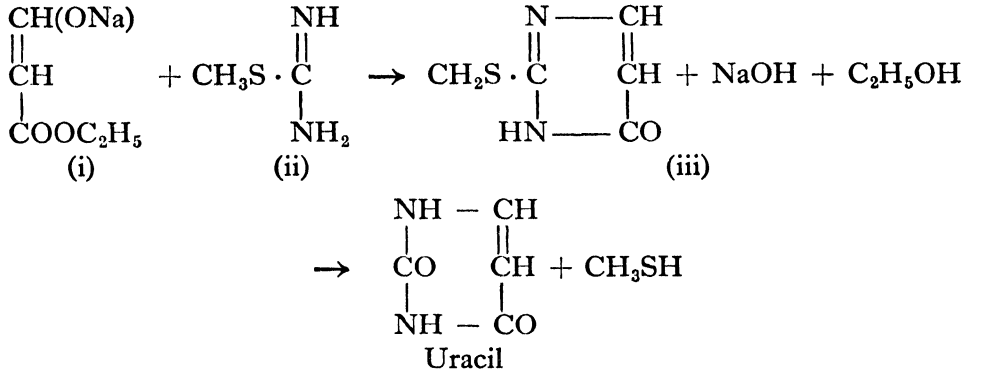
* Liebig and Wöhler, *Ann.*, 1838, **26**, 276.
† Biltz, Heyn, and Bergius, *Ann.*, 1916, **413**, 68, 76.
‡ *J. physiol. Chem.*, 1900, **31**, 162. § *Ber.*, 1901, **34**, 3761.

acetic acid on this compound a product was obtained which gave uracil on heating with pyridine:



By a series of similar reactions thymine was obtained from methylacrylic acid.

Wheeler and Merriam* found that the sodium derivative of formylacetic ester (i) condenses with γ-methylthiocarbamide† (ii) to form 2-methylthiol-4-oxypyrimidine (iii), from which uracil is easily formed by saponification with boiling mineral acid:



In a similar way they obtained thymine by using formylpropionic ester in place of formylacetic ester, while acetoacetic ester gave 4-methyluracil, and methylacetoacetic ester gave dimethyluracil. Carboxyl derivatives have been prepared by replacing the hydroxyacrylic esters by ethoxylmethylene malonic ester or by formylsuccinic ester. A great variety of pyrimidine derivatives have been obtained by means of these reactions.‡

Somewhat later Wheeler and Liddle§ found that more satisfactory results could be obtained, in the preparation of uracil, by using thiocarbamide in place of γ-methylthiocarbamide. The intermediate 2-thiouracil is quantitatively converted into uracil when boiled with an aqueous solution of chloracetic acid. In a

* *Amer. Chem. J.*, 1903, **29**, 480.

† γ-Thiocarbamides are obtained by the action of alkyl halides on thiourea. They are strongly basic and, as a rule, undergo condensations very readily.

‡ For a list of researches on pyrimidine compounds down to 1908 see *Amer. Chem. J.*, 1908, **40**, 250. Since that time a large number of papers have appeared, notably by Wheeler, Johnson, and Johns.

§ *Ibid.*, 1908, **40**, 547.

similar way Wheeler and MacFarlane substituted thiocarbamide for γ-methylthiocarbamide in the preparation of thymine, and carried out the reaction in alcoholic solution. The intermediate 2-thiothymine was converted into thymine by boiling with a solution of monochloracetic acid:

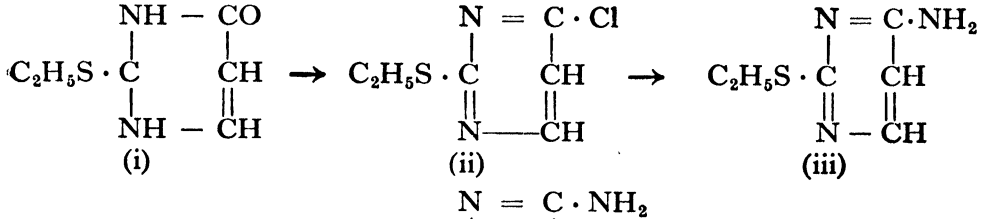
2-Thiothymine → Thymine

Uracil crystallizes from water in fine, colourless needles. With diazobenzene sulphonic acid it gives a red solution, while with bromine water and excess of baryta, a purple or violet coloured precipitate and solution are obtained.

Thymine crystallizes from water in colourless needles or rosettes which dissolve readily in hot water. With diazobenzene sulphonic acid it gives a more intense red colour than uracil, but it does not behave like uracil towards bromine water and baryta. Like uracil, it combines with silver nitrate, forming a compound which is precipitated by ammonia and dissolved by an excess of this reagent.

Cytosine.—This base was obtained in 1893 by Kossel and Neumann by the hydrolysis of thymus nucleic acid with 40 per cent sulphuric acid under pressure. The correct constitutional formula was assigned to this base by Kossel and Steudel in 1903.*

Wheeler and Johnson † prepared 2-ethyl-thiol-6-chloropyrimidine (ii) by the action of phosphorus pentachloride on 2-ethyl-thiol-6-oxypyrimidine (i). On boiling this substance with alcoholic ammonia the corresponding amide (iii) was formed, which, in turn, was converted by saponification into 2-oxy-6-aminopyrimidine, identical with cytosine:

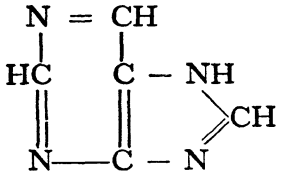


(i) (ii) (iii)

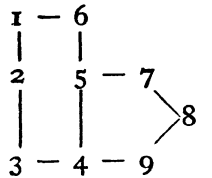
Cytosine

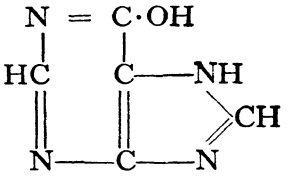
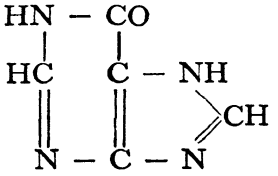
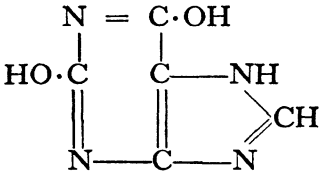
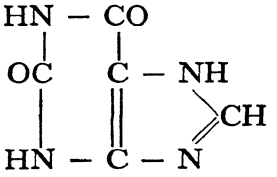
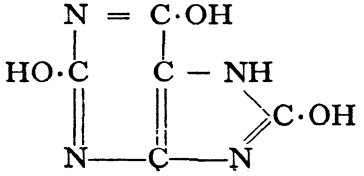
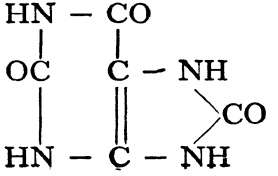
* *Zeit physiol. Chem.*, 1903, **38**, 49. † *Amer. Chem. J.*, 1903, **29**, 505.

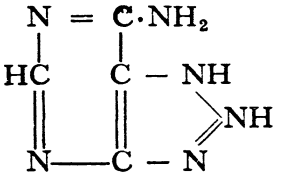
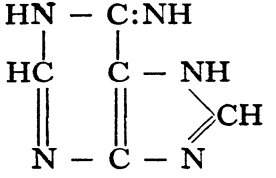
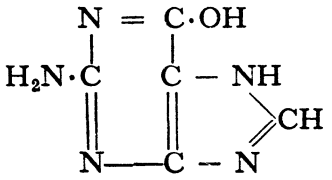
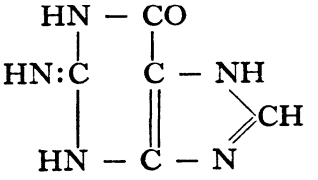
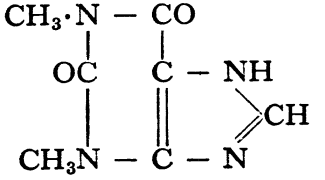
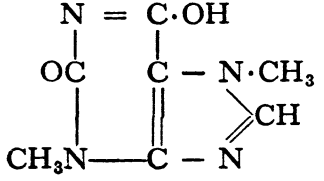
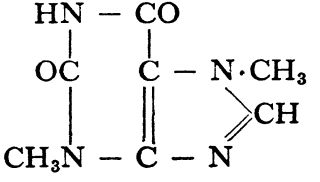
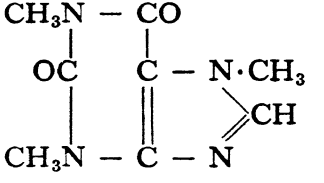
THE PURINE BASES



Purine



	A. Common Name and Empirical Formula.	B. Structural Formula with Minimum Saturation.	C. Systematic Name Corresponding to B.	D. Structural Formula with Maximum Saturation.	E. Systematic Name Corresponding to D.
1	Hypoxanthine, $C_5H_4ON_4$.		6-Hydroxy-purine.		6-Oxypurine-dihydride (1 : 6).
2	Xanthine, $C_5H_4O_2N_4$.		2 : 6-Dihy-droxypurine.		2 : 6-Dioxypurine-tetrahydride (1 : 2 : 3 : 6).
3	Uric acid, $C_5H_4O_3N_4$.		2 : 6 : 8-Trihy-droxypurine.		2 : 6 : 8-Trioxy-purine-hexahydride (1.2.3.6.7.8).

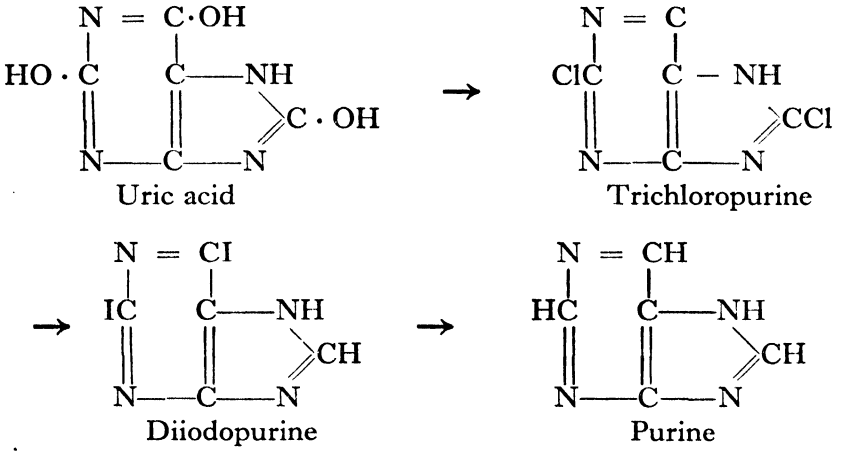
4	Adenine, $C_5H_5N_5$.		6-Amino-purine.		6-Iminopurine-dihydride (1 : 6).
5	Guanine, $C_5H_5ON_5$.		2-Amino-6-hydroxypurine.		2-Imino-6-oxy-purine-tetrahydride (1.2.3.6).
6	Theophylline (1 : 3-Dimethylxanthine), $C_7H_8O_2N_4$.				1 : 3-Dimethyl-2 : 6-dioxypurine-tetrahydride (1.2.3.6).
7	Theobromine (3 : 7-Dimethylxanthine), $C_7H_8O_2N_4$.		3 : 7-Dimethyl-6-hydroxy-2-oxopurine-dihydride (2:3).		1 : 7-Dimethyl-2 : 6-dioxypurine-tetrahydride (1.2.3.6).
8	Caffeine (1 : 3 : 7-Trimethylxanthine), $C_8H_{10}O_2N_4$.				1:3:7-Trimethyl-2 : 6-dioxypurine-tetrahydride (1.2.3.6).

Cytosine crystallizes in colourless plates containing one molecule of water of crystallization, which it loses at 100°. It forms salts with acids and is converted into uracil on treatment with nitrous acid. With dilute acidified solutions of potassium bismuth iodide it gives a brick-red crystalline precipitate. It behaves like uracil towards diazobenzene sulphonic acid or bromine water and baryta. Cytosine forms an easily crystallizable picrate.

THE PURINE BASES

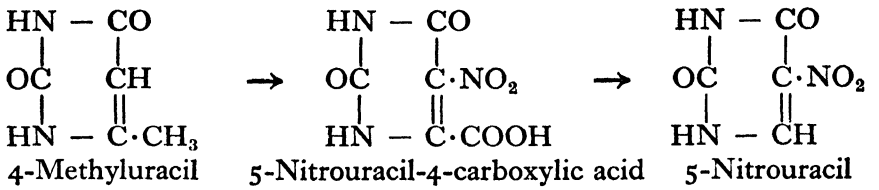
From the table on pp. 198, 199 it is evident that the purine or xanthine bases may be regarded as derivatives of one parent substance to which the term "purine" (*purum uricum*) was applied by Emil Fischer. Purine itself does not appear to be present in the living organism.

Purine has been synthesized by Fischer.* For this purpose uric acid is heated with an excess of phosphorus oxychloride at 160°, when a crystalline compound, trichloropurine, is obtained. This substance, by successive reduction with hydriodic acid and zinc dust, is converted into diiodopurine and thence into purine.

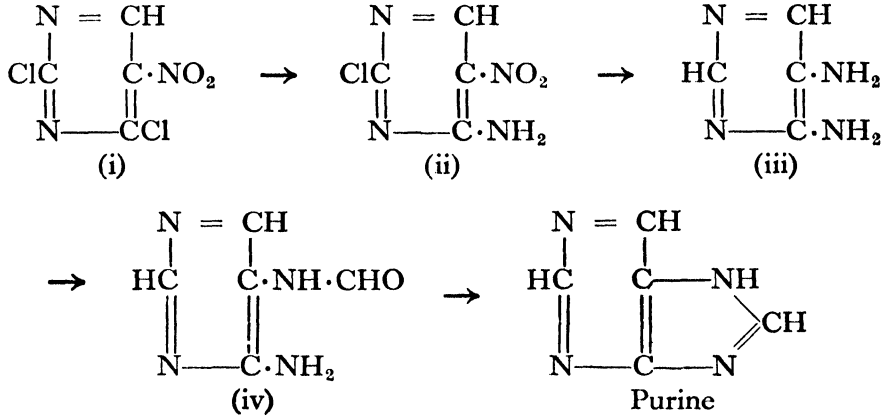


Isay † has carried out a direct synthesis of purine. The starting point is 4-methyluracil, which results from the hydrolysis of the condensation product of urea and acetoacetic ester. On treatment with nitric acid a nitro group is introduced, and the methyl group is oxidized to carboxyl. On boiling this product 5-nitrouracil is obtained:

* *Ber.*, 1899, 32, 493. † *Ber.*, 1906, 39, 280.



When heated with phosphorus oxychloride under pressure, 5-nitrouracil yields 2:4-dichloro-5-nitropyrimidine (i), which with ammonia gives 2-chloro-4-amino-5-nitropyrimidine (ii). On reduction with hydriodic acid, 4:5-diaminopyrimidine (iii) is produced. This is converted into its formyl derivative (iv), which gives purine when heated at 210°.



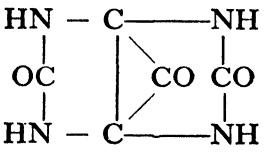
The Constitution of Uric Acid.—As uric acid is found to break up on oxidation into equimolecular proportions of alloxan and urea according to the equation

$$\underset{\text{Uric acid}}{C_5H_4N_4O_3} + O + H_2O = \underset{\text{Alloxan}}{C_4H_2N_2O_4} + \underset{\text{Urea}}{CO(NH_2)_2}$$

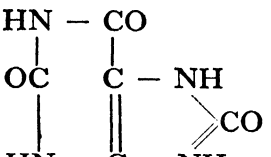
its molecule presumably contains the nuclei of both these substances. Since all four hydrogen atoms of uric acid are replaceable by metals, it is probable that these hydrogen atoms are linked to the nitrogen atoms as in the alloxan molecule. That this is actually the case is proved by the elimination of the whole of the nitrogen as trimethylamine when tetramethyluric acid is hydrolyzed with concentrated hydrochloric acid at 70°.

Two formulæ have been proposed which agree equally well with the above reactions, the first by Fittig* and the second by Medicus.†

* *Ber.*, 1878, **11**, 1792. † *Ann.*, 1875, **175**, 236.



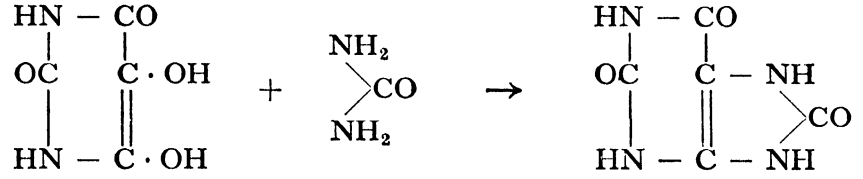
(i)



(ii)

Fittig's formula represents uric acid as a symmetrical arrangement of two condensed pyrimidine nuclei, while that of Medicus consists of a fused pyrimidine and glyoxaline (iminazole) nucleus. Now Fischer prepared two monomethyluric acids, one of which gave methylalloxan and urea on oxidation, while the other gave alloxan and methylurea. This can only be accounted for by the grouping proposed by Medicus, and this formula has been established by synthesis.

Synthesis of Uric Acid.—Following an observation of Strecker that uric acid could be hydrolyzed at high temperatures with the production of ammonia, carbon dioxide, and glycocoll, Horbaczewski obtained uric acid by heating urea with glycocoll or with cyanacetic acid. The yield, however, was very small, and the synthesis gives little information as to the structure of uric acid. In 1888 Behrend and Roosen obtained uric acid by the condensation of isodialuric acid (prepared by a long and complicated process) with urea in the presence of strong sulphuric acid:

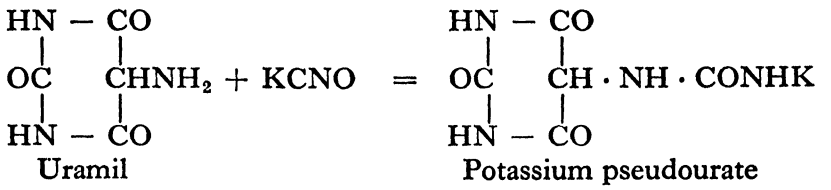


Isodialuric acid

The most satisfactory syntheses are those discovered by Emil Fischer and by W. Traube, and these may be briefly described.

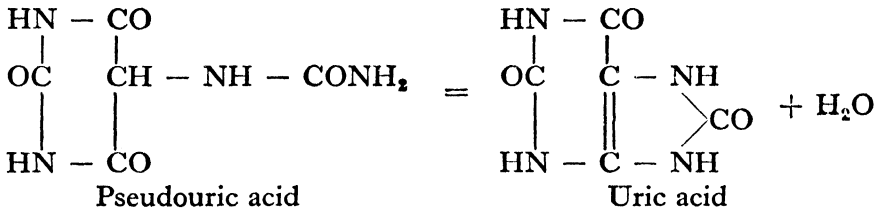
*Emil Fischer's Synthesis.**—As early as 1838, Liebig and Wöhler had correctly surmised the relation of uramil to uric acid; but their attempts to unite uramil and cyanic acid were unsuccessful. By boiling uramil with a solution of potassium cyanate, Baeyer and Schlieper † obtained the potassium salt of pseudouric acid, but they were unable to dehydrate the resulting acid.

* *Ber.*, 1897, **30**, 559. † *Ann.*, 1863, **127**, 3.



Uramil — Potassium pseudourate

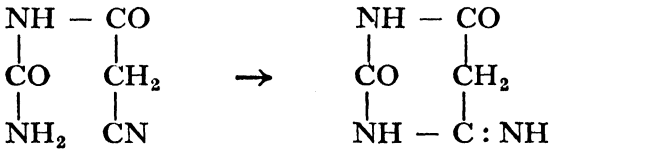
Fischer found that pseudouric acid may be dehydrated to uric acid by means of molten oxalic acid, or, more simply, by boiling with dilute mineral acids.



Pseudouric acid — Uric acid

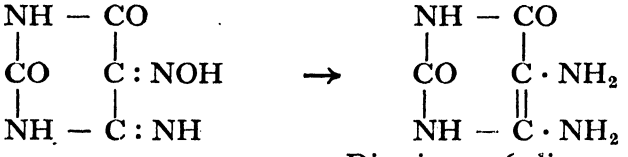
This method has been applied by Fischer to the synthesis of various alkyluric acids and, indirectly, to several of the xanthine bases.

*W. Traube's Synthesis.**—Cyanacetylurea is first prepared by the condensation of cyanacetic acid or its ester and urea in the presence of phosphorus oxychloride, and the product is then converted into 4-amino-2 : 6-dioxypyrimidine by the action of alkalies.



Cyanacetylurea — 4-Amino-2 : 6-dioxypyrimidine

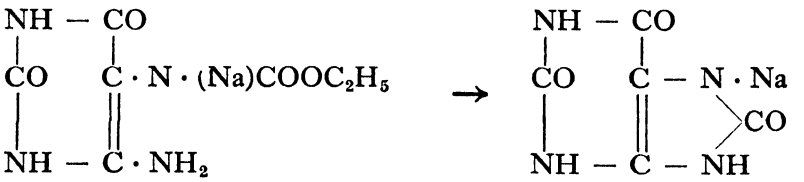
By the action of nitrous acid the hydrogen of the methylene group is replaced by an isonitroso group, and the latter on reduction is transformed into an amino group:



4 : 5-Diamino-2 : 6-dioxypyrimidine

By the action of chloroformic ester a urethane is obtained, and when the sodium derivative of the latter is heated, alcohol is eliminated and sodium urate is formed.

* *Ber.*, 1900, 33, 1371, 3035.



Sodium urate

This method has been extended to embrace certain of the xanthine bases.

In addition to Fischer's synthetic method, a number of methyluric acids * may be obtained by the direct methylation of uric acid. For this purpose the silver or lead salts of uric acid may be treated with methyliodide, or they may be obtained, more conveniently, by the action of methylsulphate in the presence of dilute caustic soda.† The positions taken up by the methyl groups depend upon the conditions of the experiment.

The Occurrence and Structure of the Purine Bases.—It has already been pointed out that guanine and adenine are primary hydrolytic products of the nucleic acids, and it would appear that these two substances are the only purine bases obtained as primary products. Three other purine bases, hypoxanthine, xanthine, and uric acid, are formed from these by metabolic processes.

With the exception of adenine, which was obtained from nucleic acid by Kossel in 1886, all these bases have long been known. In addition, theobromine, which is a constituent of cocoa beans (*Theobroma cacao*), and caffeine, which occurs in small quantities in tea and coffee, were studied by Stenhouse as early as 1843.

The elucidation of the structure of the purine or xanthine bases is almost entirely due to Emil Fischer, and the earlier work need not be discussed here. Fischer‡ made an elaborate study of the degradation products of the caffeine molecule, but as the constitution of this substance has since been established by its synthesis, the story of this work has lost some of its earlier interest.

The decomposition of caffeine into dimethylalloxan and methylurea indicates a structure similar to that of uric acid, and ultimately the constitutional formula shown on p. 199 was adopted. Fischer observed that xanthine was converted into alloxan and urea on oxidation, and that theobromine was obtained by the action of methyliodide on the lead salt of xanthine, while caffeine resulted from the methylation of theobromine. The conversion of guanine

* For the preparation and structure of several of the methyluric acids see E. Fischer (*Ber.*, 1899, **32**, 461).

† Biltz and Damm, *Ann.*, 1916, **413**, 186. ‡ *Ann.*, 1882, **108**, 141.

into xanthine, by the action of nitrous acid, was first observed by Strecker.

The Synthesis of the Purine Bases.—The synthesis of the purine bases has attracted a number of chemists, but it will be sufficient to review briefly the methods adopted by Fischer and Traube.

Fischer's Methods.—From the close relationship between the purine bases and uric acid, it is evident that at least three methods for the conversion of the latter into the former present themselves:

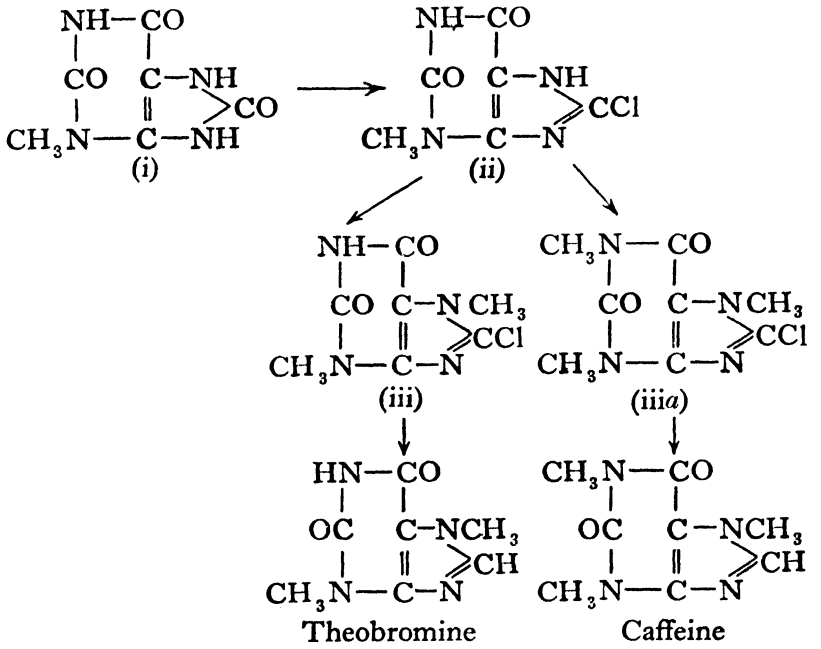
1. Reduction of uric acid to xanthine and subsequent methylation.

2. Methylation of uric acid to monomethyluric acid, reduction to methylxanthine, and further methylation.

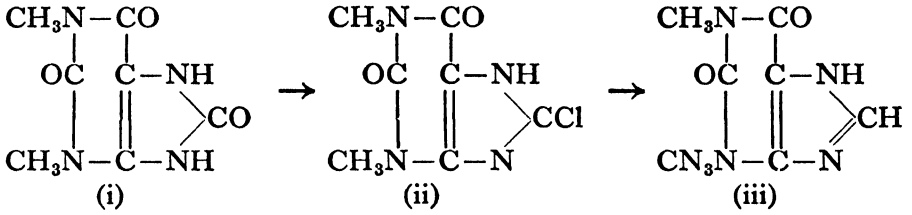
3. Further methylation of uric acid to di- and tri-methyluric acids, and reduction of the products to the corresponding methylxanthines.

A few examples of these methods may be briefly reviewed.

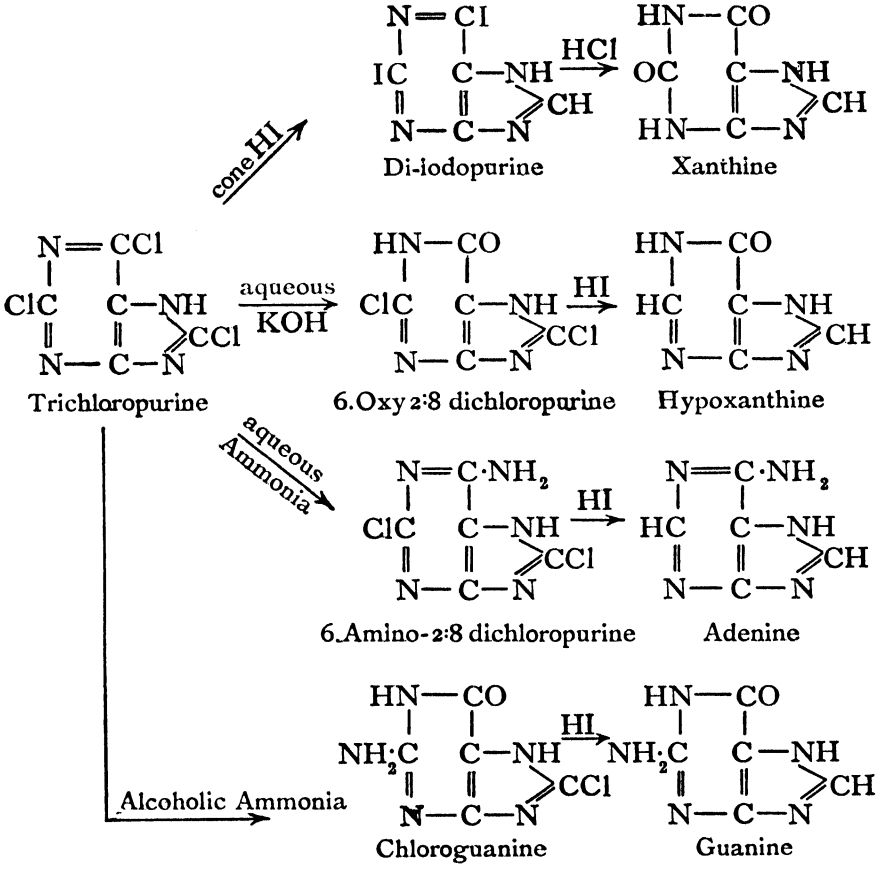
(*a*) By the direct methylation of uric acid, 3-methyluric acid (i) may be obtained, and this is converted into 3-methyl-8-chloroxanthine (ii) by the action of phosphorus oxychloride. The latter can be methylated to give 8-chlorotheobromine (iii) or 8-chloroxanthine (iii*a*), which are readily converted into theobromine and caffeine respectively:



(*b*) When heated with a mixture of phosphorus pentachloride and oxychloride, 1 : 3-dimethyluric acid (i) yields 8-chlorotheophylline (ii), which on reduction is converted into theophylline (iii):



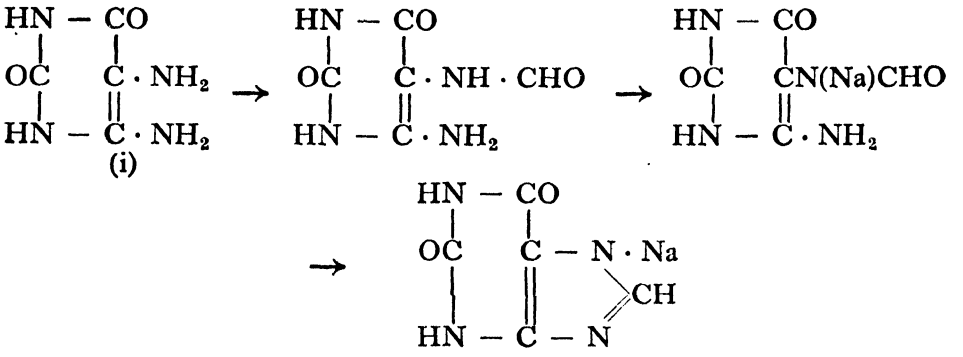
In addition to purine itself a number of the purine bases may be obtained from trichloropurine by means of the following reactions:



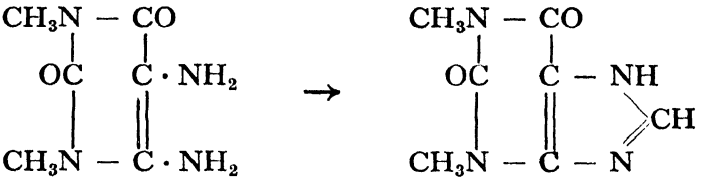
The conversion of trichloropurine into purine has already been described (p. 200).

Traube's Method.—The synthesis of uric acid starting from urea and cyanacetic acid has already been described. The sodium deriva-

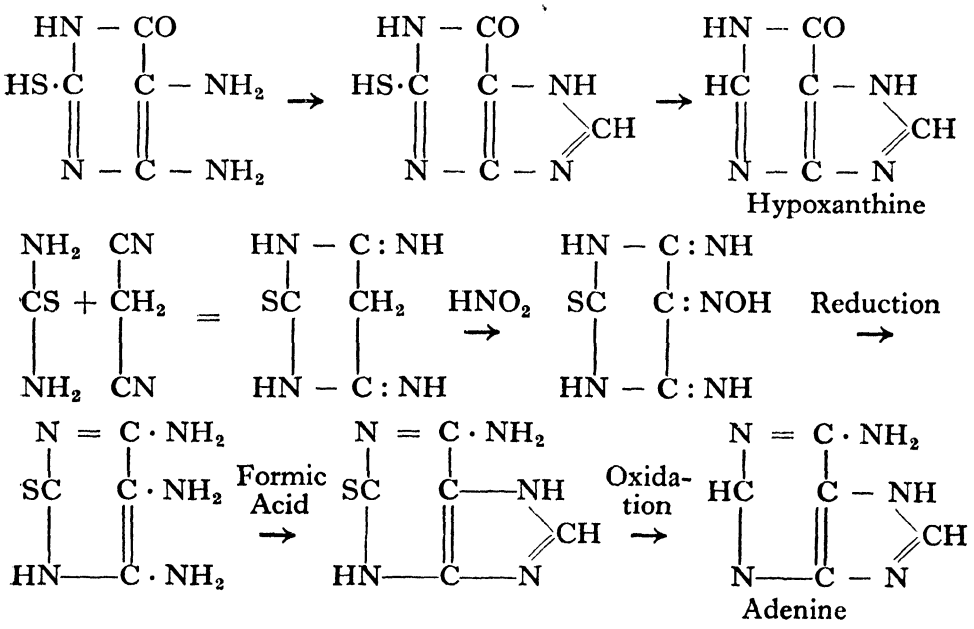
tive of xanthine is obtained when 4 : 5-diamino-2 : 6-dioxo-pyrimidine (i) is transformed into its formyl derivative, by means of formic acid, and the sodium derivative of the latter is heated:



When dimethylurea is used in place of urea, theophylline is obtained:

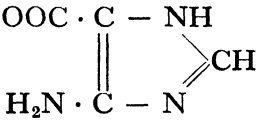


This method is capable of considerable extension, e.g. monomethylurea yields 3-methylxanthine, and guanidine gives guanine. When thiourea is employed and the sulphur ultimately removed with dilute nitric acid, hypoxanthine is obtained, while adenine may be synthesized starting from thiourea and methylene cyanide:



In addition to the foregoing methods Fischer and Ach * have shown that theophylline, xanthine, and several other purine bases may be obtained by the demethylation of caffeine.

Before concluding our study of the purine derivatives it is advisable to draw attention to the fact that in all these syntheses it has been customary to start with pyrimidine derivatives or substances which condense to form pyrimidine compounds at an early stage of the synthesis. In view of the suggestion of F. G. Hopkins † that the naturally occurring purine bases originate from histidine (p. 145), it is noteworthy that Fargher and Pyman ‡ proposed to prepare xanthine by the condensation of 4-amino-glyoxaline-5-carboxylic acid,

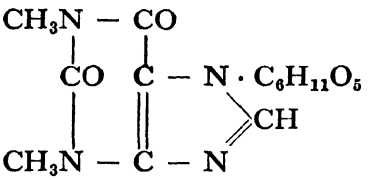


with cyanic acid and subsequent elimination of water. The attempted preparation of this glyoxaline derivative was not successful, but it should be remembered that the chemistry of the glyoxaline nucleus has only attracted considerable attention within the last few years.

SYNTHETIC NUCLEOSIDES AND NUCLEOTIDES

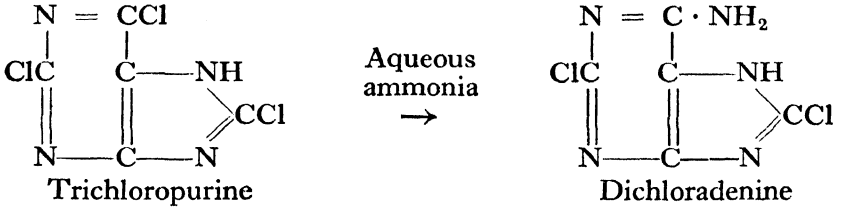
It has already been stated that the compound of sugar and base which remains when a nucleic acid is incompletely hydrolyzed is termed a nucleoside. Several such nucleosides—or purine glucosides, as they may also be termed—have been synthesized by Emil Fischer and his collaborators. For this purpose acetobromglucose (p. 59) or an allied compound is condensed with the silver salt of one of the purines in xylene solution. For example, the silver salt of theophylline and acetobromglucose give tetra-acetyl-theophylline-*d*-glucoside $[C_7H_7O_2N_4 \cdot C_6H_7O_5(COCH_3)_4]$.§ For the removal of the acetyl groups, this substance, in methylalcoholic solution, is treated with ammonia at 0°, and the resulting compound crystallized from methylalcohol in vacuo. In this way theophylline-*d*-glucoside, probably having the formula

* *Ber.*, 1906, **39**, 423.
† *Trans.*, 1906, **109**, 628.
‡ *Trans.*, 1919, **115**, 217.
§ Fischer and Helferich, *Ber.*, 1914, **47**, 210.

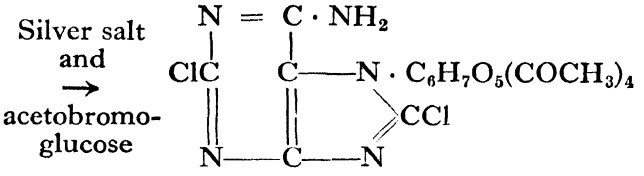


is obtained.

The glucosides of hypoxanthine, xanthine, guanine, and adenine have been obtained indirectly from trichloropurine and dichloradenine. For this purpose it is better to use dichloradenine in place of trichloropurine, because in the subsequent removal of the acetyl groups ammonia reacts with the chlorine in the latter case. The preparation of adenine-*d*-glucoside and hypoxanthine-*d*-glucoside is illustrated by the equations:

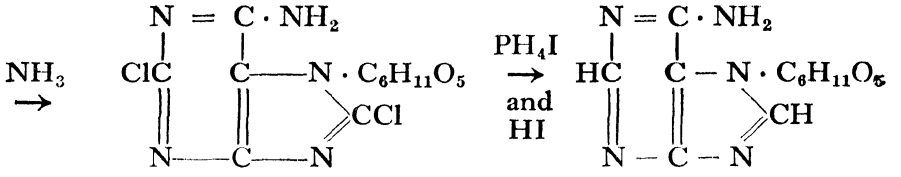


Trichloropurine — Aqueous ammonia → Dichloradenine



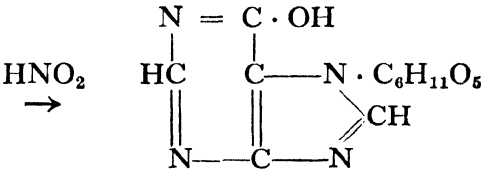
Silver salt and → acetobromo-glucose

Dichloradenine-tetra-acetyl-*d*-glucoside



NH_3 → Dichloradenine-*d*-glucoside

PH_4I and HI → Adenine-*d*-glucoside



HNO_2 →

Hypoxanthine-*d*-glucoside

In a similar manner purine galactosides and rhamnosides have been obtained.*

* Fischer and Fodor, *Ber.*, 1914, 47, 1058; Fischer, *Ber.*, 1914, 47, 1377.

The synthesis of the nucleotides, necessitating of course the introduction of a phosphoric acid radicle, presented considerable experimental difficulty. Success was eventually achieved by the use of phosphorus oxychloride. When this is condensed with theophylline-*d*-glucoside in pyridine solution at -20° a good yield of theophylline-*d*-glucoside phosphoric acid is obtained.*

$$[C_7H_7O_2N_4 \cdot C_6H_9O_5 \cdot HPO_2] \cdot 2H_2O$$

Purine residue — Glucose residue — Phosphoric acid residue

The actual configuration of this compound has not yet been determined.

These results serve to illustrate the progress which has been made in the problem of the synthesis of the nucleic acids, and to show that the achievement of this object will demand an experimental technique of the very highest order.

REFERENCES.

Nucleic Acids, Their Chemical Properties and Physiological Conduct, by W. Jones: Monographs on Biochemistry (London, 1913).

Synthesen in der Puringruppe, by E. Fischer (*Ber.*, 1899, **32**, 436).

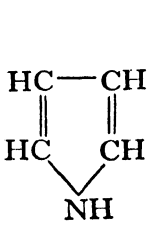
Vegetable Alkaloids, by A. Pictet, trans. by H. Biddle (New York, 1904).

* Fischer, *Ber.*, 1914, **47**, 3193.

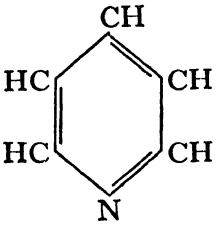
CHAPTER X

The Alkaloids

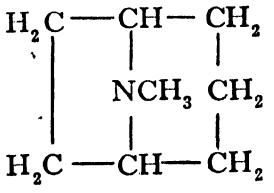
Taken in its etymological sense, the term alkaloid may serve to designate all organic substances which possess basic properties, but it is obviously impracticable to consider the many diverse types of organic bases as alkaloids. In recent years alkaloids have been defined as those organic bases which contain a cyclic nitrogenous nucleus, and which are formed in the organism of the plant. Even this limited definition is too wide for our purpose, since it would include many of the bases already dealt with, and in this book we shall consider the term alkaloid to cover those natural organic bases which may be regarded as derivatives of pyrrole, pyridine, tropane, norharman, quinoline, isoquinoline, and phenanthrene.



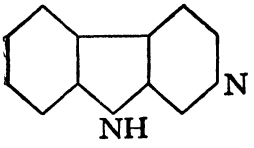
Pyrrole



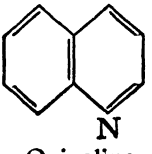
Pyridine



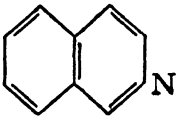
Tropane



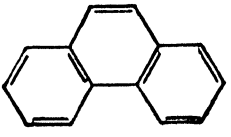
Norharman



Quinoline



Isoquinoline


Phenanthrene

In 1806 Sertürner, an apothecary of Hanover, obtained a basic crystalline body from opium, and thus had the honour of discovering the first vegetable base. In 1817 he published a second paper which bore the title, " Ueber das Morphium, eine neue salzfähige Grundlage und die Mekonsäure als Hauptbestandthiele des Opiums ", in which

he definitely characterized morphium as a vegetable "alkali" and compared its behaviour with that of ammonia. This paper aroused considerable interest, and during the next twenty-five years many more alkaloids were discovered.

These vegetable bases were regarded as substituted ammonias by Liebig and Hofmann, while the latter considered most of them to be tertiary bases; but little further progress was made until the discovery of pyridine, quinoline, and isoquinoline, and the recognition of these compounds as frequently forming the fundamental skeletons of many of the alkaloid molecules. In 1834 Runge obtained from coal tar a basic substance, C_9H_7N, which he termed "leucol", and between 1846 and 1851 Anderson examined bone oil and isolated a homologous series of volatile bases from it, of which the first member, of the formula C_5H_5N, was termed "pyridine". Meanwhile Gerhardt, in 1842, had distilled quinine, cinchonine, and strychnine with solid caustic potash and had obtained an oil which he termed "quinoline", and which Hofmann showed was identical with Runge's "leucol". Subsequently several other alkaloids, such as nicotine, conine, piperine, &c., were converted into pyridine or one of its homologues by heating with zinc dust. In a similar way isoquinoline, which was discovered in coal tar by Hoogewerff and van Dorp in 1885, has been shown to be related to papaverine, narcotine, hydrastinine, and berberine.

The subsequent progress in the isolation, investigation, and synthesis of the alkaloids has been very rapid, and before the individual alkaloids are described a short sketch of the occurrence, isolation, and general lines of investigation of the alkaloids may be considered.

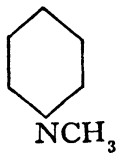
Occurrence of the Alkaloids.—The alkaloids are not confined to any special orders or parts of plants, but they are specially abundant in the families of *Rubiaceæ*, *Solanaceæ*, and *Papaveraceæ*, and rare in those of *Labiatæ* and *Rosaceæ*. As a rule several closely related alkaloids are present in the same plant, as, for example, opium—from which more than twenty individual alkaloids have been obtained. The alkaloids rarely exist in the plant in the free state, but are more frequently present as lactates, malates, citrates, or tannates, or combined with some other acid which is a peculiar accompaniment of the alkaloid.

Extraction of the Alkaloids.—The extraction of alkaloids from plants, and their subsequent purification, are frequently operations of considerable difficulty, partly because in many cases two or more alkaloids occur together, and partly because soluble neutral and

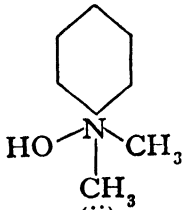
acidic substances, such as the glucosides, sugars, tannins, malic acid, &c., are present in large quantity. As a rule the alkaloids may be extracted by treating the macerated plant or vegetable product with dilute acids, which convert the alkaloids into soluble salts. The filtered solution may then be treated with soda to liberate the alkaloids, which, being sparingly soluble, are usually precipitated and may be separated by filtration; if not, the alkaline solution is extracted with ether, chloroform, &c. In a few cases, such as the extraction of nicotine from tobacco, water may be used as the extracting solvent.

Many of the alkaloids give insoluble precipitates with a solution of tannic, picric, or phosphomolybdic acid, with platinic chloride, or with a solution of mercuric iodide in potassium iodide. These, and several other reagents, are often used for their detection and isolation.

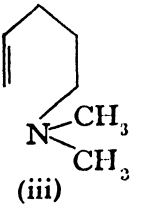
Investigation of the Alkaloids.—The alkaloids contain one or two atoms of nitrogen, rarely more, and are usually tertiary bases. The nitrogen is usually firmly fixed in the molecule, but it can occasionally be removed as ammonia by the action of strong reducing agents. By the action of alkalies the nitrogen is sometimes removed as methylamine, indicating the attachment of a methyl group to the nitrogen atom in the molecule. The stability of the cyclic nitrogen atom is greatly diminished by making the element quinquevalent, and this property has been utilized by Hofmann for breaking down the molecule by so-called " exhaustive methylation ". The application of this method to piperidine may be considered as an example. Piperidine is converted into the tertiary base (i), and this forms an additive compound with methyliodide, which gives dimethylpiperidinium hydroxide (ii) on treatment with silver oxide. The latter, on distillation, loses water and gives the unsaturated open-chain base (iii).



(i)



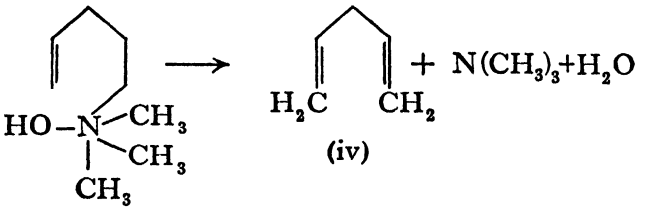
(ii)



(iii)

When this compound is again submitted to a similar series of reactions and distilled, piperylene (iv), trimethylamine, and water are formed,

the reaction being analogous to the decomposition of tetraethylammonium hydroxide into triethylamine, ethylene, and water.

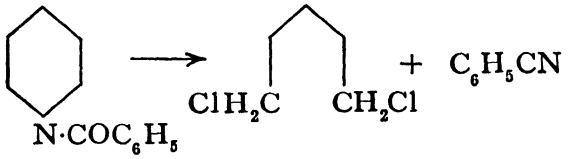


(iv)

$$(C_2H_5)_4N \cdot OH \rightarrow C_2H_4 + (C_2H_5)_3N + H_2O$$

The introduction of an acid radicle, e.g. $-COOC_2H_5$, $-COC_6H_5$, in place of a hydrogen atom attached to a cyclic nitrogen atom, renders the compound readily oxidizable. This has already been illustrated in the case of benzoylpiperidine (p. 151).

Von Braun has employed phosphorus pentachloride for breaking down the cyclic structure of several alkaloids;* e.g. benzoylpiperidine treated with phosphorus pentachloride gives a mixture of benzonitrile and 1 : 5-dichloropentane:



More recently the same author has introduced cyanogen bromide for the same purpose.†

When oxygen is present in the alkaloid it is usually in the form of hydroxyl or methoxyl, and occasionally as carboxyl or an ester group. It is a remarkable fact that by far the greater number of alkaloids contain one or more methoxyl groups (OCH_3). The method employed for the estimation of these groups—called the Zeisel method—depends on the fact that all substances containing methoxyl groups are decomposed by hydriodic acid, yielding methyliodide and a hydroxy compound in accordance with the general equation:

$$n(-OCH_3) + nHI = n(-OH) + nCH_3I$$

and by estimating the methyliodide obtained (by conversion into silver iodide), the number of methoxyl groups can be determined.

When hydrolysis can be effected it should precede any other process of decomposition. The action of alkalies, zinc dust, and

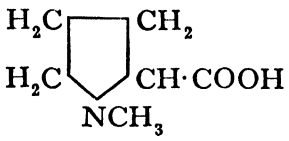
* *Ber.*, 1904, 37, 3588. † *Ber.*, 1916, 49, 2624.

other reducing agents often yield useful results, but the most valuable information is usually derived by regulated oxidation of the alkaloids.

THE PYRROLE ALKALOIDS

The following alkaloids may be considered as derivatives of pyrrole: hygrine, betonicine and turicine, and stachydrine.

Hygrine is found to the extent of about 0·2 per cent in Peruvian cusco leaves, and was isolated from this source by Liebermann in 1889. It is a liquid, and, like the majority of alkaloids, is lævorotatory. It is ketonic and a tertiary base containing a N-methyl group. On oxidation it yields hygric acid, which on dry distillation decomposes into carbon dioxide and N-methylpyrrolidine. A possible structure of hygric acid is therefore



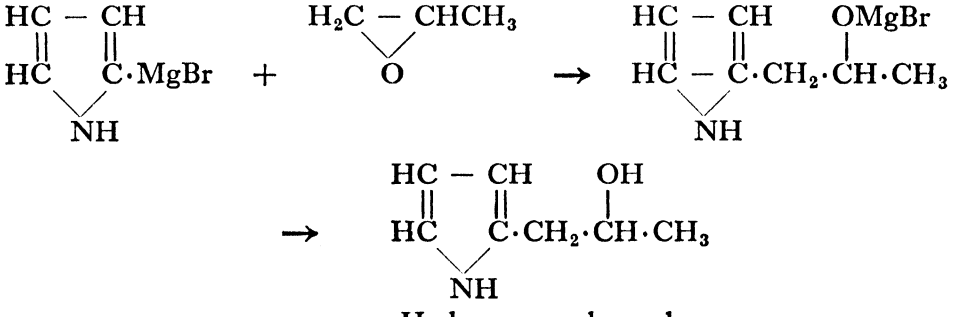
Hygric acid

and this structure has been established by its synthesis by the action of methylamine on $\alpha\delta$-dibromopropylmalonic ester,

$$[CH_2Br \cdot CH_2 \cdot CH_2 \cdot CBr(COOC_2H_5)_2]$$

by Willstätter and Ettlinger.*

Hygrine has been obtained synthetically by Hess † as follows: pyrrole magnesium bromide reacts with propylene oxide to give hydroxypropylpyrrole:

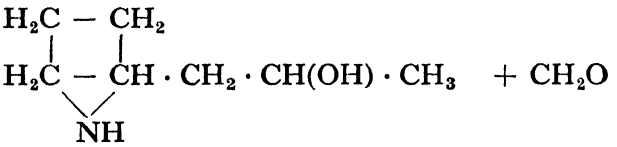


Hydroxypropylpyrrole

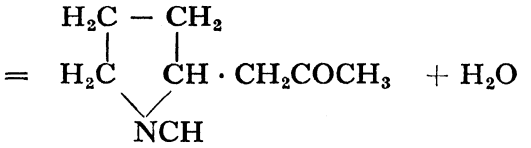
On reduction the corresponding pyrrolidine compound is obtained. The latter is then methylated by the action of formaldehyde,

* *Ann.*, 1901, 326, 91. † *Ber.*, 1913, 46, 4104.

which at the same time oxidizes the carbinol group to the ketone and gives *r*-hygrine:

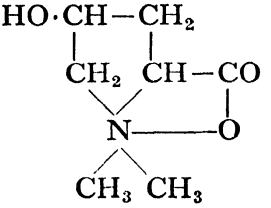


Hydroxypropylpyrrolidine

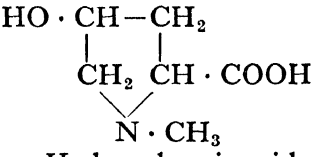


* *r*-Hygrine

Betonicine and **Turicine** are respectively the lævo and dextro varieties of a base of the formula $C_7H_{13}O_3N$. Both bases have been shown to occur in the Betony † (*Betonia officinalis*). Both these bases have been obtained by Küng ‡ by the methylation of oxyproline (p. 145), so that they probably have the constitution:



This formula has been recently confirmed by the work of Goodson and Clewer,§ who obtained 4-hydroxyhygric acid from the bark of *Croton gubouga*, and on methylation of the acid obtained a mixture of betonicine and turicine:

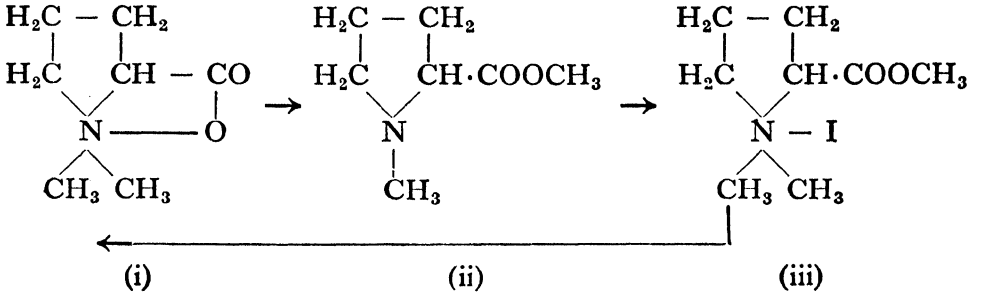


4-Hydroxyhygric acid

Stachydrine, $C_7H_{13}O_2N$, occurs in the leaves of the orange tree and in the tubers of *Stachys tuberifera*, and was first isolated by von Planta in 1890. This compound is frequently classified as an alkaloid, but Schultze and Trier || consider that the base is derived from proline (p. 145). On distillation stachydrine yields the isomeric

* *r* = racemic. † Schultze and Trier, *Zeit. physiol. Chem.*, 1912, **79**, 235.
‡ *Ibid.*, 1913, **85**, 217. § *Trans.*, 1919, **115**, 923.
|| *Zeit. physiol. Chem.*, 1909, **59**, 233.

methyl ester of hygric acid (ii), and the methiodide of this ester (iii) is transformed into stachydrine (i) on hydrolysis: *

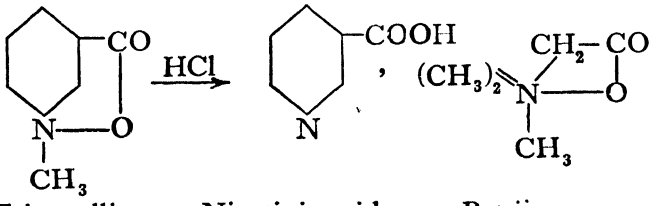


(i) (ii) (iii)

THE PYRIDINE ALKALOIDS

Trigonelline, piperine, conine, and nicotine may be considered as four of the simpler pyridine alkaloids.

Trigonelline, $C_7H_7O_2N$, was isolated from the seeds of fenugreek (*Trigonella fœnum græcum*) by Jahns in 1885, and two years later he established its constitution by observing that when heated with hydrochloric acid to 270° it is converted into nicotinic acid. The natural product was found to be identical with the "methylbetaïne" of nicotinic acid previously synthesized by Hantzsch.†



Trigonelline Nicotinic acid Betaïne

On account of the presence of a betaïne ring, trigonelline may also be considered as a derivative of betaïne.

Piperine occurs to the extent of 5 to 9 per cent in the dried fruits of black and white pepper (*Piper nigrum*), from which it was first isolated by Oersted in 1819. It is a tasteless, colourless, weak base, and has no action on polarized light. On hydrolysis with alcoholic potash it is converted into piperidine and piperic acid:

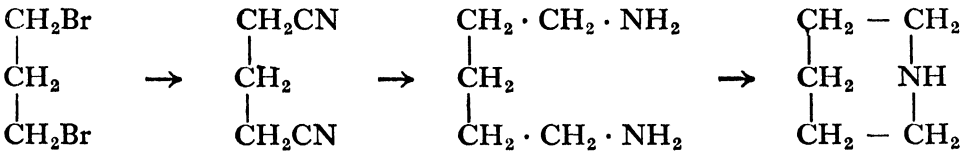
$$C_{17}H_{19}O_3N + H_2O = \underset{\text{Piperidine}}{C_5H_{11}N} + \underset{\text{Piperic acid}}{C_{12}H_{10}O_4}$$

and as Rügheimer ‡ showed that piperic chloride reacts with piperidine

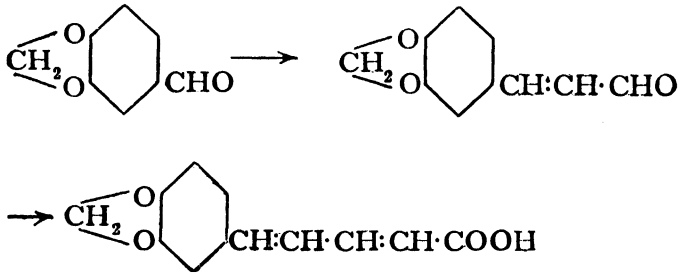
* Trier, *Zeit. physiol. Chem.*, 1910, 67, 324.
† *Ber.*, 1886, 19, 31. ‡ *Ber.*, 1852, 15, 1390.

to give piperine, the alkaloid may be regarded as an amide of piperic acid.

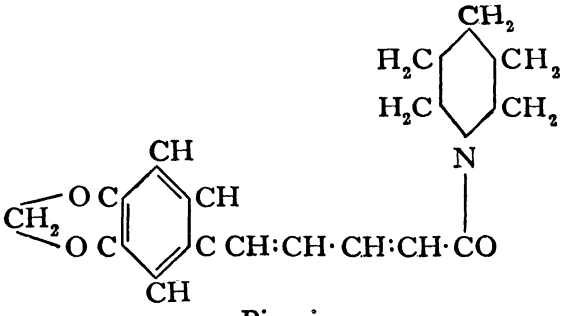
Piperidine was obtained by Ladenburg * by distilling the hydrochloride of pentamethylene-diamine, which in turn may be obtained from trimethylene-dibromide:



Piperic acid was investigated by Fittig and obtained synthetically by Ladenburg and Scholtz.† Piperonal condenses with acetaldehyde in the presence of caustic soda solution to give piperonylacrolein, and this is converted into piperic acid by Perkin's reaction:



The complete structure of piperine is therefore represented by the formula:

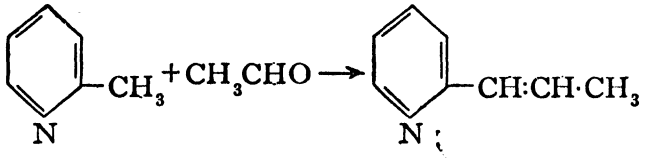


Piperine

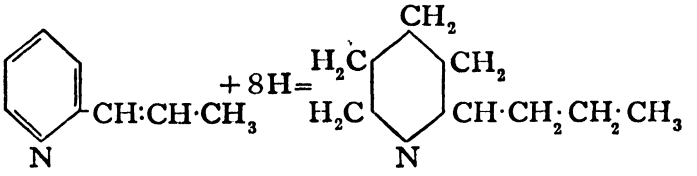
Conine, $C_8H_{17}N$, was first isolated as the free base from hemlock by Giesecke in 1827. The hemlock (*Conium maculatum*) contains three principal alkaloids, conine, coniceïne, and conhydrine, together with smaller quantities of other bases. These alkaloids are present in combination with malic and caffeic acids. Conine

* *Ber.*, 1885, **18**, 2956, 3100. † *Ber.*, 1894, **27**, 2958.

was examined by Liebig and Gerhardt, and the correct empirical formula derived by Hofmann in 1881. It is a volatile, oily base, and is extremely poisonous. Hofmann's work had shown that in all probability conine was α-propylpiperidine. Ladenburg heated pyridine propiodide and obtained a mixture of bases, one of which appeared to be γ-propylpyridine, since on oxidation it gave pyridine-γ-carboxylic acid. These bases were subsequently shown to be isopropylidene derivatives. On reduction of each compound with sodium and alcohol he obtained products which resembled conine, but neither was identical with it. In 1886 Ladenburg condensed picoline (α-methylpyridine) with paracetaldehyde in sealed tubes with the aid of zinc chloride and obtained allyl-pyridine. On reduction with sodium and alcohol *r*-conine was obtained. The base was resolved by crystallizing its acid tartrate, and the dextrorotatory form, on heating, was identical with conine. This alkaloid which had caused the death of the wisest of men was the first to succumb to the synthetic skill of the chemist.

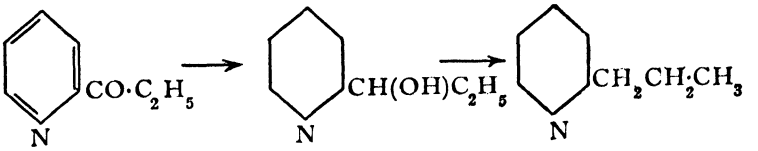


α. Picoline α-Allyl pyridine



r. Conine

Conine was subsequently synthesized by Engler and Bauer.* By distilling molecular equivalents of the calcium salts of propionic and picolinic acids they obtained α-ethylpyridyl-ketone, which on complete reduction gave *r*-conine.

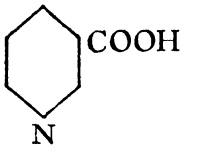


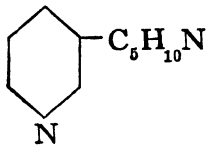
α-Ethylpyridyl-ketone α-Ethylpyridyl-alkamine *r*-Conine

* *Ber.*, 1891, **24**, 2530; 1894, **27**, 1775.

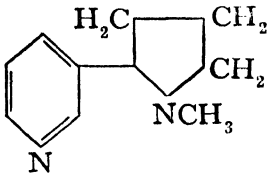
Nicotine.—This alkaloid was first isolated from the leaves of the tobacco plant by Posselt and Reimann in 1828. For a long time it was considered to be the only alkaloid present in tobacco, but more recently Pictet and Rotschy * and others have shown that several closely related alkaloids are present. The nicotine content of tobacco varies from 0·6 to 10 per cent, and in general the better grades of tobacco contain the smaller amounts of the alkaloid.

Nicotine is a diacid base, and since it forms two isomeric methiodides with methyliodide it is also a ditertiary base. On oxidation with chromic acid, nicotinic acid (pyridine-β-carboxylic acid) is obtained, so that nicotine is a β derivative of pyridine.



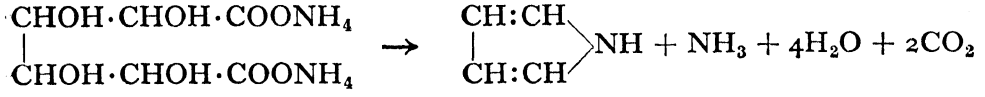


Pinner first put forward the correct constitutional formula for nicotine (β-pyridyl-α-N-methylpyrrolidine), and the correctness of his view was confirmed by the synthesis of nicotine by Pictet, Crépieux, and Rotschy.†

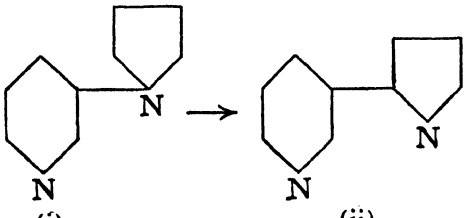


Nicotine

The Synthesis of Nicotine.—When the mucate of β-amidopyridine is submitted to dry distillation, N-β-pyridylpyrrole (i) is obtained, the reaction being exactly analogous to the formation of pyrrole by the distillation of ammonium mucate:



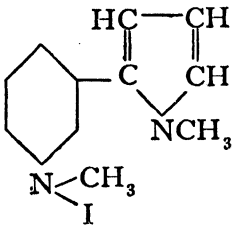
On passing the vapours of N-β-pyridylpyrrole through a tube heated to a dull red heat, an intramolecular change takes place and αβ-pyridylpyrrole is obtained (ii):



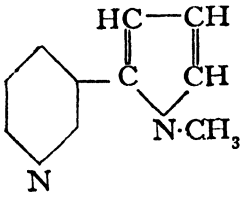
(i) (ii)

* *Ber.*, 1901, 34, 696. † *Ber.*, 1895, 28, 1904; 1904, 37, 2018.

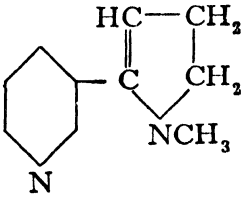
The potassium salt of the latter reacts with methyliodide to give the methiodide of N-methyl-$\alpha\beta$-pyridylpyrrole (iii), which gives nicotyrine (iv) on distillation over lime:



(iii)



(iv)



(v)

The selective reduction of the pyrrole nucleus could not be accomplished in one stage. The iodo derivative of nicotyrine on reduction with tin and hydrochloric acid gives dihydronicotyrine, which may possibly have the structure (v). The perbromide of the latter then yields *i*-nicotine on similar reduction. The alkaloid was resolved by the fractional crystallization of the tartrate, when the lævo form was found to be identical with the natural product, and much more poisonous than the dextro form.

THE TROPANE GROUP

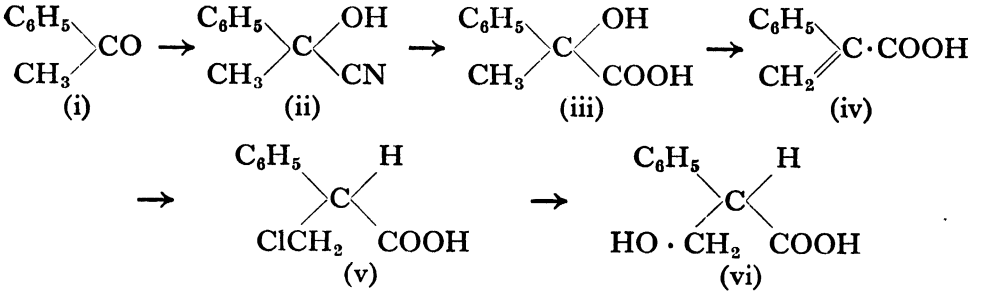
Several plants of the Solanaceæ family are characterized by the presence in their tissues of some very poisonous alkaloids, which in their chemical properties and constitution closely resemble one another. These plants are the belladonna (*Atropa belladonna*), the henbane (*Hyoscyamus niger*), the common stramonium (*Datura stramonium*), and different species of the genus Scopolia. The bases which are present have been separated from each other with difficulty, but the presence of the following alkaloids has been certainly established: atropine, hyoscyamine, pseudohyoscyamine, and hyoscine, which are all isomers of the formula $C_{17}H_{33}NO_3$; belladonnine, $C_{17}H_{21}NO_2$, and scopolamine (atroscine), $C_{17}H_{21}NO_2$, about which little is known.

Of these seven alkaloids, atropine, hyoscyamine, and scopolamine are found in all the above plants, while belladonnine has only been found in the deadly nightshade (*Atropa belladonna*) up to the present.

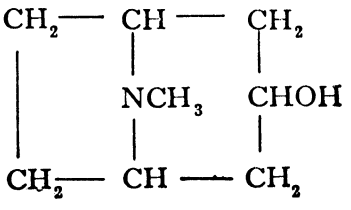
Atropine and its Allies.—Atropine was discovered in 1831 in the roots of the belladonna almost simultaneously by Mein and by Geiger and Hesse. Its chief use in medicine depends upon its action in dilating the pupil and paralysing the accommodation of the eye.

On hydrolysis with acid or alkali it yields an acid—tropic acid—and a base—tropine.

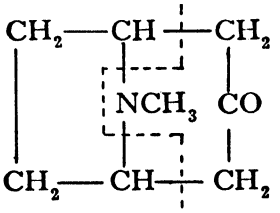
Tropic Acid.—The correct constitutional formula for tropic acid was derived by Kraut, and a successful synthesis first achieved by Ladenburg and Rügheimer in 1880.* These chemists used acetophenone as the starting point of the synthesis, but as the method has been superseded by more satisfactory methods in recent years we need not consider it in detail. M'Kenzie and Wood † convert acetophenone (i) into its cyanhydrin (ii), which on hydrolysis gives atrolactinic acid (iii), and this on distillation under reduced pressure, atropic acid (iv). On treatment with ethereal hydrochloric acid the chloro acid (v) is obtained, which gives tropic acid (vi) on treatment with sodium carbonate solution:



Tropine.—Our knowledge of the constitution of tropine is chiefly due to the work of Ladenburg and Merling, and more recently to the researches of Willstätter. Tropine is a secondary alcohol which on oxidation gives the ketone, tropinone. The relation between these compounds is made clear by their respective structural formulæ:



Tropine



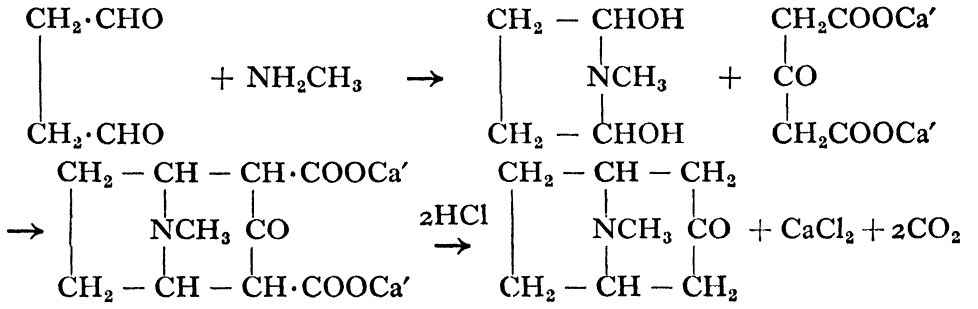
Tropinone

The synthesis of tropinone by Willstätter ‡ is classical on account of the richness of the field explored.

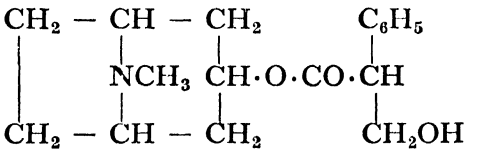
By imaginary hydrolysis of the tropinone molecule at the dotted lines, succindialdehyde, methylamine, and acetone are obtained,

* *Ber.*, 1880, **13**, 373, 2041. † *Trans.*, 1919, **115**, 828.
‡ *Ber.*, 1901, **34**, 129, 3163; *Ann.*, 1901, **317**, 307, 1903, **326**, 1.

and Robinson * has succeeded in obtaining tropinone by the condensation of these substances in aqueous solution. An improved yield was obtained when the calcium salt of acetone-dicarboxylic acid was employed instead of acetone. This synthesis is important not only on account of its simplicity, but also by reason of its bearing on Robinson's views on the phytochemical synthesis of the alkaloids.

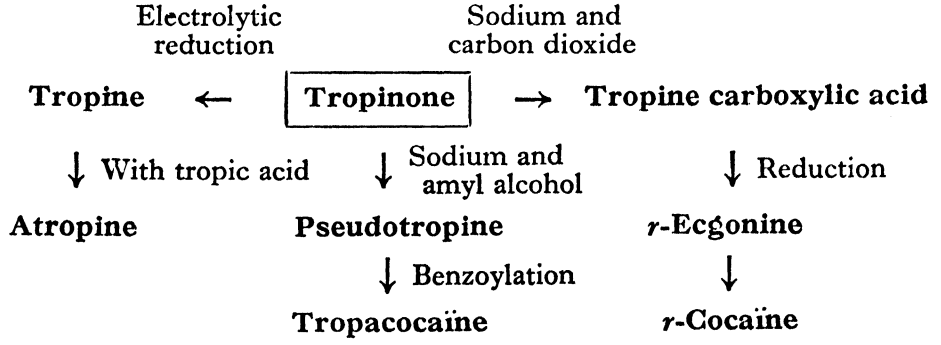


Since hyoscyamine and atropine are stereoisomeric they may be represented by the formula:



According to Gadamer † the tropine in both atropine and hyoscyamine is inactive, and the only difference between the two alkaloids lies in the fact that lævotropic acid is present in the molecule of hyoscyamine.

The relationship between tropinone and several closely allied alkaloids may be briefly summarized:



When tropine is heated with sodium amyloxide it is converted into a tropine identical with the pseudotropine obtained by the

* *Trans.*, 1917, **111**, 762. † *Arch. d. Pharm.*, 1902, **239**, 294.

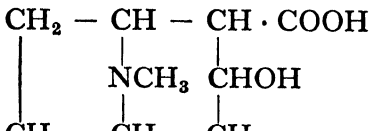
hydrolysis of the coca alkaloid tropacocaïne. Tropine and pseudotropine are thus isomeric, and Barrowcliff and Tutin * have shown that the isomerism is dependent on molecular asymmetry, i.e. it is cistrans isomerism.

Cocaïne and some Synthetic Substitutes.—Cocaïne, along with several closely related alkaloids, occurs in coca leaves (*Erythroxylon coca*), from which it was first isolated by Neumann in 1860. It had long been known that the South American Indians were in the habit of chewing these leaves as a stimulant. Cocaïne is used in medicine usually in the form of its hydrochloride, as a rapid local anæsthetic.

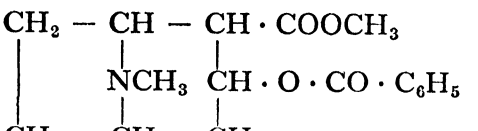
Cocaïne is a tertiary base, and on hydrolysis it yields ecgonine, benzoic acid, and methylalcohol:

$$C_{17}H_{21}NO_4 + 2H_2O = C_9H_{15}NO_3 + C_7H_6O_2 + CH_3OH$$

The preparation of ecgonine from tropinone has already been mentioned, and the relation of the former to cocaïne is made clear by the formulæ,



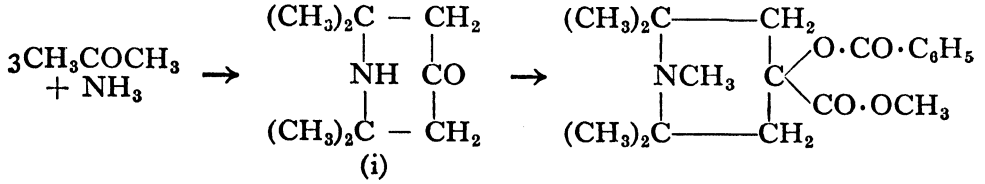
Ecgonine



Cocaïne

On account of the fact that cocaïne solutions become mouldy on long standing and decompose on boiling, various attempts have been made to prepare suitable substitutes. The benzoyl derivative of pseudotropine is known as *tropacocaïne*. It is a stronger local anæsthetic, less toxic and more resistant to micro-organisms than cocaïne.

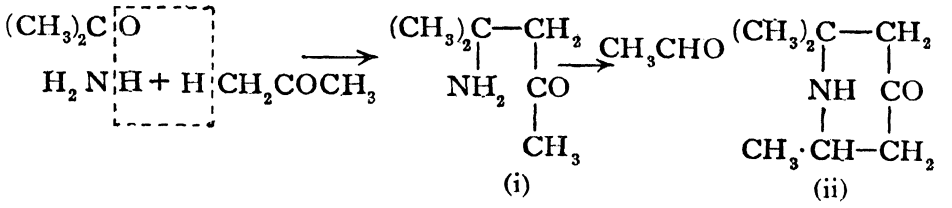
In 1897 Merling obtained triacetonamine (i) by the condensation of three molecules of acetone with one of ammonia. It is a crystalline solid with an ammoniacal and somewhat camphoraceous odour. On hydrolysis of the cyanhydrin, followed by benzoylation and methylation of the resulting acid, *α-eucaine* is obtained:



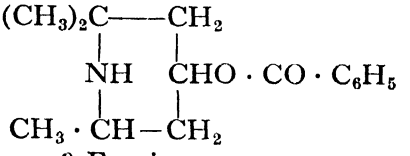
(i)

* *Trans.*, 1909, 95, 1966.

On account of the irritant action of α-eucaine it has been largely superseded by *β-eucaine*. Diacetonamine (i) is obtained by the condensation of two molecules of acetone and one of ammonia, and this on further condensation with acetaldehyde gives vinyldiacetamine (ii):

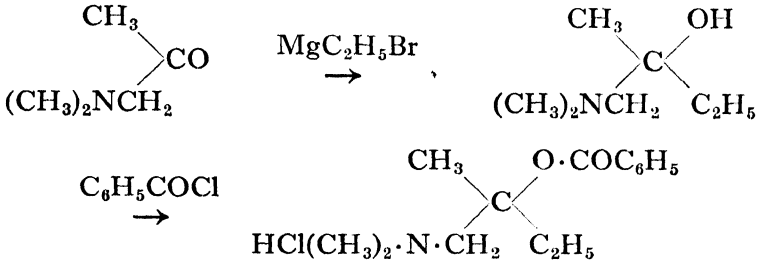


On reduction to the corresponding alcohol, two isomerides are obtained. The higher melting isomeride on benzoylation gives *β-eucaine*:

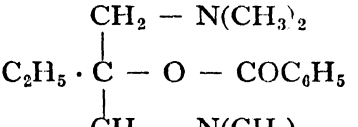


β-Eucaine

Stovaine is a well-known local anæsthetic for minor surgical operations. It is prepared from dimethylaminoacetone as follows:



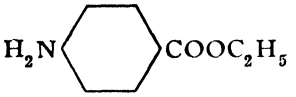
Alypine is similar in constitution to stovaine and is frequently administered along with heroin (diacetyl-morphine), since it enhances the demulcent and sedative effects of the latter:



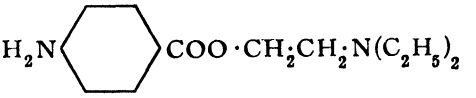
Alypine

Anæsthesine, one of the simplest local anæsthetics, is obtained

by reducing ethyl-*p*-nitrobenzoate with tin and hydrochloric acid. Its diethylamino derivative is known as *novacaïne*,*



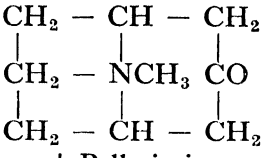
Anæsthesine



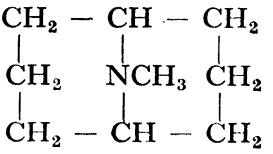
Novacaïne

THE POMEGRANATE ALKALOIDS

The root bark of the pomegranate contains a number of alkaloids, amongst which pelletierine and the isomeric *iso*pelletierine, $C_8H_{15}NO$, *pseudo*pelletierine, $C_9H_{15}NO$, and the two isomeric methylpelletierines, $C_9H_{17}NO$, may be mentioned. *Pseudo*pelletierine is interesting because it resembles tropinone and gives rise to an eight-carbon ring on exhaustive methylation. It has been shown that this alkaloid is a higher homologue of tropinone. The parent compound is known as granatinine:



ψ-Pelletierine



Granatinine

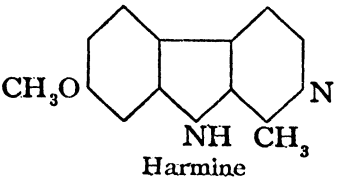
NORHARMAN ALKALOIDS

Harmine and Harmaline.—These two alkaloids, which have been the subject of much investigation during the last few years, occur together in the seeds of *Peganum harmala*. Their respective molecular formulæ are $C_{13}H_{12}ON_2$ and $C_{13}H_{14}ON_2$. Since 1885 O. Fischer and his co-workers have published several papers dealing with harmine, but in view of the fact that the earlier constitutional formulæ have been discredited, only the more recent work need be considered.

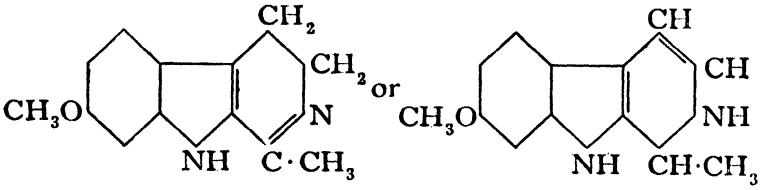
In 1919 Perkin and Robinson † proposed the following formulæ:

* For the more recent study of the anæsthetic action of the tropine derivatives see von Braun and Müller (*Ber.*, 1918, **51**, 741).

† *Trans.*, 1919, **115**, 933.

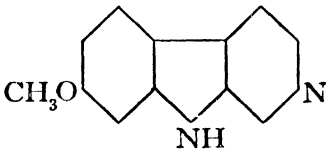


Harmine

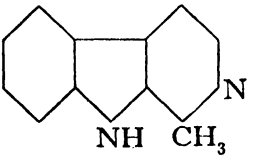


Harmaline

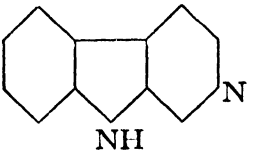
By the elimination of the methyl, methoxyl, and methoxyl and methyl groups from the harmine molecule, norharmine, harman, and norharman or 4-carboline are formed respectively:



Norharmine



Harman

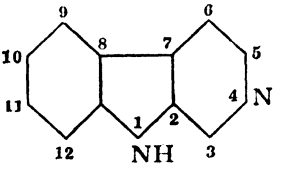


Norharman (4-carboline)

Norharman may therefore be regarded as the parent of these compounds, and it will be observed that it contains a fused benzene-pyrrole-pyridine nucleus.* Harman has been obtained by the oxidation of tryptophane (p. 145) by Hopkins and Cole,† and Späth ‡ has shown that harman is identical with the alkaloid aribine from *Aratiba rubra.*

The suggestion that harmine is a methyl-methoxy-4-carboline has been more recently confirmed by Kermack, Perkin, and Robinson,§ by its degradation to norharman by two separate methods, and the

* Perkin and Robinson (*Trans.*, 1919, **115**, 970) suggest the name "carboline" for this structure, indicating an analogy both to carbazole and quinoline.

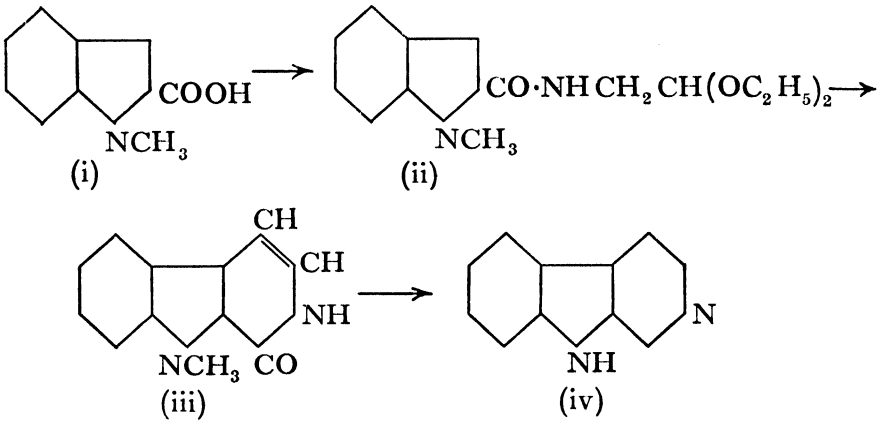


Thus harmine is 11-methoxy-3-methyl-4-carboline.

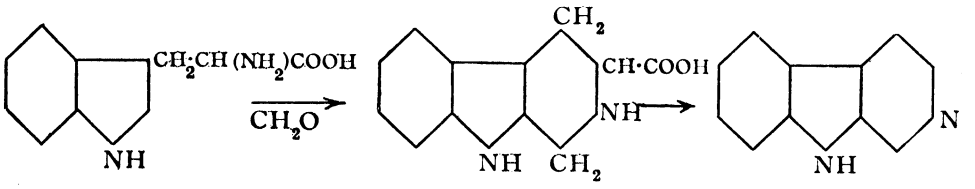
† *J. Physiol.*, 1903, **29**, 451. ‡ *Chem. Zeit.*, 1919, **43**, 555.

§ *Trans.*, 1921, **119**, 1602.

synthesis of the latter compound. For this purpose 1-methylindole-2-carboxylic acid (i) is converted by the condensation of its chloride with amino-acetal into 1-methylindole-2-carboxy-acetalylamide (ii), which, when treated with alcoholic hydrochloric acid, furnishes 5-keto-4-5-dihydroindole diazine (iii), from which norharman (N) is obtained by distillation with zinc dust:



Norharman also results from the condensation of tryptophane with formaldehyde in the presence of sulphuric acid followed by oxidation of the product:



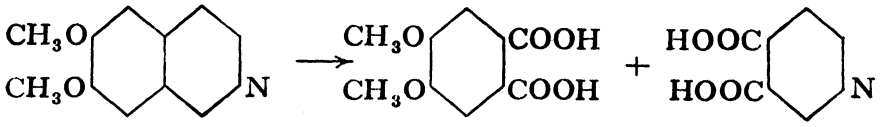
THE ISOQUINOLINE ALKALOIDS

The alkaloids of this group comprise the opium alkaloids papaverine, laudanosine, narcotine, narceïne, cryptopine, and protopine, together with the two alkaloids, hydrastine and berberine, which occur in the roots of golden seal (*Hydrastis canadensis*), and others of less importance. It should be noted that more than twenty alkaloids have been isolated from opium, and in addition the dried sap of the poppy contains resins, gums, sugars, fats, and protein matter. Of these alkaloids the following may be briefly considered: papaverine, laudanosine, and hydrastine.

Papaverine.—This alkaloid was first isolated from commercial

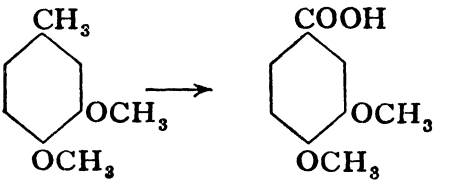
narcotine by Merck in 1848, and it has attracted the attention of a considerable number of chemists.

Papaverine is a tertiary base and is optically inactive. On treatment with hydriodic acid it loses four methoxyl groups, while on fusion with caustic potash it gives two compounds, only one of which contains nitrogen. The compound containing nitrogen may be oxidized to metahemipinic and cinchomeronic acids, which prove that it is dimethoxy-isoquinoline:



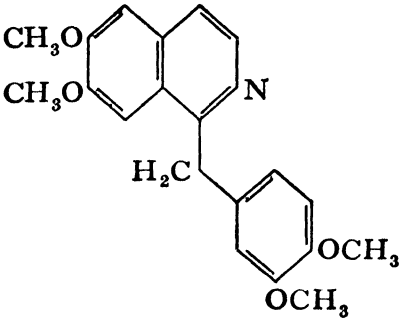
Dimethoxy-isoquinoline | *m*-Hemipinic acid | Cinchomeronic acid

The product containing no nitrogen is dimethyl-homocatechol, since it gives dimethyl-protocatechuic acid on oxidation:



Dimethyl-homocatechol | Protocatechuic acid

Papaverine is represented by the formula,



Papaverine

and the correctness of this structure has been confirmed by the complete synthesis by Pictet and Gams.* The steps in this synthesis may be briefly represented as follows:

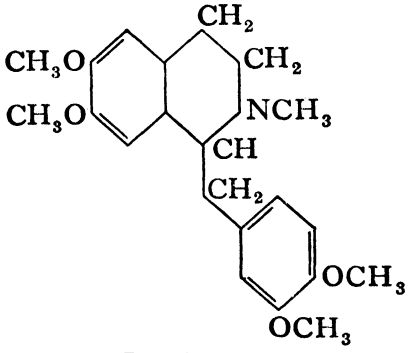
* *C. r.*, 1909, **149**, 210; *Ber.*, 1909, **42**, 2943.

Vanillin
$(CH_3O)(OH)C_6H_3CHO$
↓
Methylvanillin
$(CH_3O)_2 \cdot C_6H_3CHO$
↓
Isoethoxy-mandelonitrile
$(CH_3O)_2C_6H_3 \cdot CH(OH)CN$
↓
Homoprotocatechuic acid
$(HO)_2C_6H_3 \cdot CH_2 \cdot COOH$
↓
Homoveratroyl chloride
$(CH_3O)_2 \cdot C_6H_3 \cdot CH_2 \cdot COCl$

Veratrol
$(CH_3O)_2C_6H_4$
↓
Acetoveratrone
$(CH_3O)_2C_6H_3 \cdot CO \cdot CH_3$
↓
Isonitroso-acetoveratrone
$(CH_3O)_2 \cdot C_6H_3 \cdot CO \cdot CH : NOH$
↓
Amino-acetoveratrone hydrochloride
$(CH_3O)_2 \cdot C_6H_3 \cdot CO \cdot CH_2 \cdot NH_2, HCl$

↓
Homoveratroyl-aminoacetoveratrone
$(CH_3O)_2C_6H_3 \cdot CH_2 \cdot CO \cdot NH \cdot CH_2 \cdot CO \cdot C_6H_3(OCH_3)_2$
↓
Homoveratroyl-hydroxy-homoveratryamine
$(CH_3O)_2C_6H_3 \cdot CH_2 \cdot CO \cdot NH \cdot CH_2 \cdot CH(OH) \cdot C_6H_3 \cdot (OCH_3)_2$
↓
Papaverine
$(CH_3O)_2C_6H_3 \cdot CH_2 \cdot C : N \cdot CH : CH \cdot C_6H_2 \cdot (OCH_3)_2$

Laudanosine.—This alkaloid was first isolated by Hesse in 1871, and it has the empirical formula $C_{21}H_{27}NO_4$. The following structural formula shows that it is closely related to papaverine:



Laudanosine

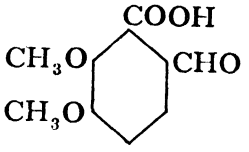
Pictet and his co-workers have synthesized laudanosine by condensing homoveratrylamine with homoveratroyl chloride and then

reducing the papaverine methochloride to methyltetrahydropapaverine. On resolution of the inactive synthetic alkaloid the dextro form was found to be identical with the natural product.

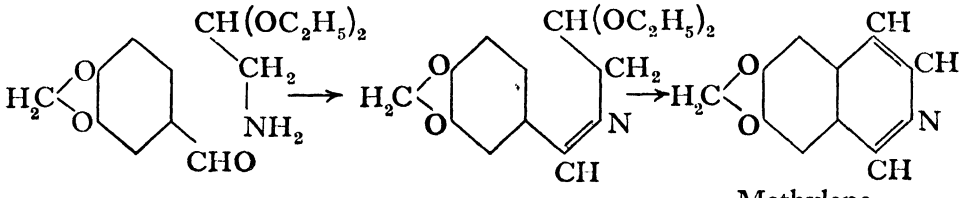
Hydrastine.—The root of the golden seal (*Hydrastis canadensis*) and the common barberry both contain hydrastine. It was first obtained in a pure condition by Perrius in 1862, and it has the empirical formula $C_{21}H_{21}NO_6$. On oxidation with potassium permanganate in acid solution it is converted into meta-opianic acid and hydrastinine,

$$C_{21}H_{21}NO_6 + H_2O + O = \underset{\text{Opianic acid}}{C_{10}H_{10}O_5} + \underset{\text{Hydrastinine}}{C_{11}H_{13}NO_3}$$

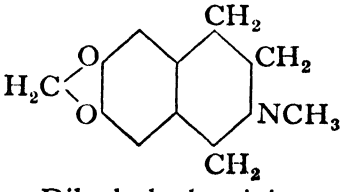
Opianic acid or 5 : 6-dimethoxy-*o*-phthalaldehydic acid has the constitution:



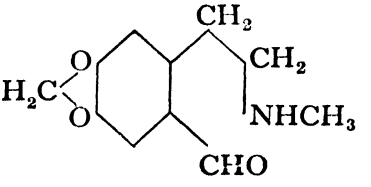
Hydrastinine may be reduced to dihydrohydrastinine, $C_{11}H_{13}NO_2$, by several methods, and Fritsch * has synthesized this compound by the action of sulphuric acid on the condensation product of piperonal and amino-acetal,



followed by the reduction of the methiodide of this compound,



Dihydrohydrastinine

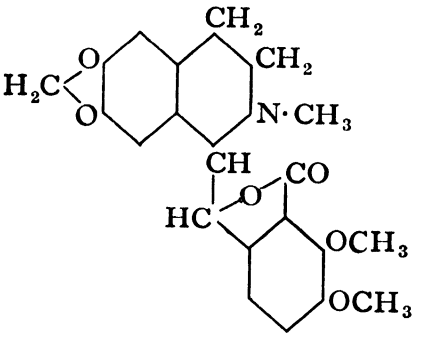


Hydrastinine

According to Freund, dihydrohydrastinine may be converted into hydrastinine by oxidation with potassium dichromate and sulphuric acid.

* *Ann.*, 1895, 286, 1.

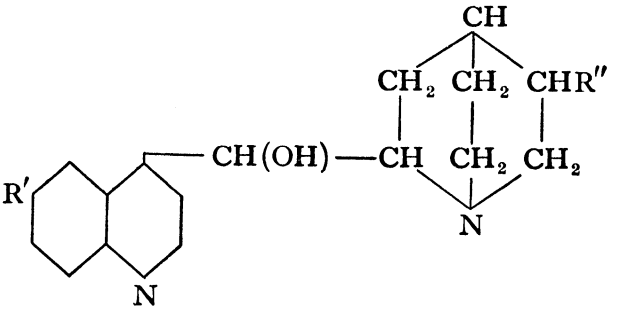
More recently hydrastinine has been synthesized by Decker and Becker.* Hydrastine has the constitution,



Hydrastine

THE QUINOLINE ALKALOIDS

The Cinchona Alkaloids, Quinine and Cinchonine.—Although cinchona bark has been used as the source of the febrifuge, quinine, since the fifteenth century, yet the two alkaloids quinine and cinchonine were not definitely isolated until 1820, when Pelletier characterized both products. Several closely related alkaloids occur in cinchona bark, and the structure of the more important of them may be represented by the formulæ:



R′.	R″.	Alkaloids.
H	· CH : CH_2	Cinchonine and cinchonidine.
H	· CH_2 · CH_3	Hydrocinchonine and hydrocinchonidine.
OCH_3	· CH : CH_2	Quinine and quinidine.
OC_2H_5	· CH_2 · CH_3	Ethylhydrocupreine and ethylhydrocupreidine.

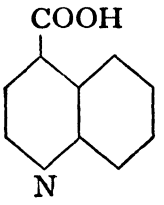
* *Ann.*, 1913, 395, 328.

Our knowledge of the constitution of these alkaloids is due to the labours of many chemists, of whom Skraup, Koenigs, von Muller, and Rabe are the more important. None of these compounds has yet been obtained synthetically.

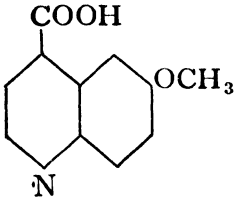
Both cinchonine and quinine are ditertiary bases, and of the two oxygen atoms in quinine one is present as hydroxyl and the other as methoxyl.

$C_{20}H_{24}N_2O_2$	$C_{19}H_{22}N_2O$
Quinine	Cinchonine

From a study of the oxidation products it is evident that each alkaloid is divisible into two parts. On oxidation with chromic acid and sulphuric acid, cinchonine yields cinchoninic acid and quinine gives quinic acid. These acids are represented by the formulæ,

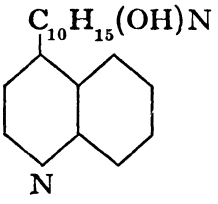


Cinchoninic acid

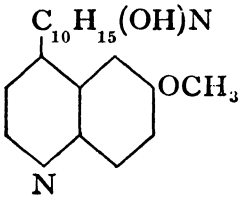


Quinic acid

so that the second half is probably identical in both alkaloids,



Cinchonine



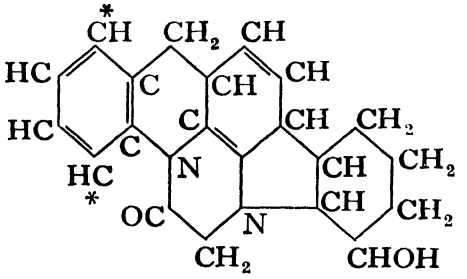
Quinine

The determination of the constitution of the second half has proved a very difficult problem. Since methods are now available for dealing with each stage of the problem of the synthesis of these alkaloids, it is probable that both compounds will be obtained synthetically in the immediate future. These researches cannot be dealt with here, and the reader should consult the excellent summaries which have appeared from time to time in the *Annual Reports of the Chemical Society*.

The therapeutic value of quinine is due to the fact that it appears to have a specific action in malaria. Many attempts have been

made to overcome the bitter taste and to obtain more soluble salts suitable for hypodermic injection. The tannate has very little taste, while esterification of the hydroxyl group with chloroformic ester, or the conversion of quinine into saloquinine by means of salicylic ester, results in the production of tasteless derivatives.

The Strychnos Alkaloids, Strychnine and Brucine.—These two alkaloids are generally found together in several species of Strychnos, the most important sources being nux-vomica seeds and Ignatius beans. These alkaloids are even more complex than quinine in structure, but Tafel and Leuchs have collected sufficient information to enable Perkin and Robinson† to propose the following formula for strychnine:



Strychnine

Brucine is the dimethoxy derivative, the two methoxyl groups replacing the hydrogen atoms attached to the two asterisked carbon atoms.

THE PHENANTHRENE ALKALOIDS

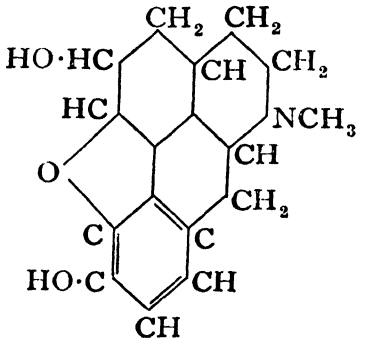
The Morphine Group.—This group includes at least four important alkaloids found in opium, namely morphine, codeïne, pseudomorphine, and thebaïne. We are still ignorant of the exact structure of any of these alkaloids, but a few of the numerous observations with regard to them may be briefly summarized.

Morphine and Codeïne have the empirical formulæ $C_{17}H_{19}NO_3$ and $C_{18}H_{21}NO_3$ respectively, indicating a difference of a methyl group between the two bases. The correctness of this view has been established by the conversion of morphine into codeïne by methylation.

Morphine is a tertiary base and contains two hydroxyl groups, one of which is phenolic and the other alcoholic. On distilla-

† *Trans.*, 1910, **97**, 309.

tion over zinc dust it gives phenanthrene, pyrrole, pyridine, trimethylamine, and ammonia. Our knowledge of the structure of the morphine molecule is largely due to the investigations of Vongerichten, Knorr, and Pschorr. The following provisional formula has been assigned to morphine by Pschorr:



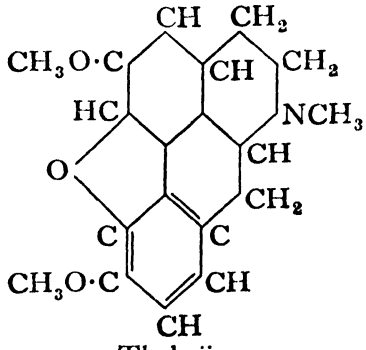
Morphine

Pseudomorphine is a non-poisonous compound which may be readily prepared from morphine by oxidation. Its structure is unknown.

Thebaïne was discovered in opium by Thiboumery in 1835. Our present knowledge of its structure is mainly due to Freund, and the following formulæ show that it is related to morphine in so far as both hydroxyl groups are methylated; but it contains two atoms of hydrogen fewer than morphine:

$(C_{16}H_{14}ONCH_3)(OH)_2$	$(C_{16}H_{12}ONCH_3)(OCH_3)_2$
Morphine	Thebaïne

Pschorr † has suggested the following formula for thebaïne:



Thebaïne

* *Ber.*, 1907, **40**, 1980. † Loc. cit.

The synthesis of substitutes for morphine, for use in medicine, has not been very successful. Methyl- and ethyl-morphine have been prepared, and the latter is stronger and exhibits a more prolonged action than codeïne. Ethylmorphine dihydrochloride is known as *dionine*. The acyl derivatives, in which the phenolic hydrogen atom is replaced by an acyl group, resemble morphine. The diacetyl derivative of morphine is known as *heroin*.

THE PHYTOCHEMICAL SYNTHESIS OF THE ALKALOIDS *

The methods which have been employed in the laboratory for the synthesis of the alkaloids bear little or no analogy to those used by the plant, and more particularly is this the case with regard to the temperatures at which the reactions are conducted by the chemist and the nature of the reagents employed. Nevertheless many of the reactions of organic chemistry, including condensation, hydrolysis, dehydration, polymerization, oxidation, and reduction, can take place under conditions of temperatures approaching those in the plant.

Gadamer † suggests that the primary products of assimilation are the same for proteins and for alkaloids. When assimilation is intense alkaloids are produced, but during periods of diminished assimilation the enzyme which synthesized proteins may break down the alkaloids, the disintegration products of which may be used in the formation of proteins.

Pictet ‡ imagines that alkaloids are produced in the plant in two successive stages, involving (1) the breakdown of complex nitrogenous substances, such as protein or chlorophyll, with the production of relatively simple basic substances; (2) the condensation of these relatively simple substances with other compounds present in the plant, with the formation of the alkaloids. Among the commonest changes in the plant are the methylation of hydroxyl or amino groups by formaldehyde,

$$R \cdot OH + CH_2O = R \cdot OCH_3 + O$$
$$R \cdot NH_2 + CH_2O = R \cdot NHCH_3 + O$$

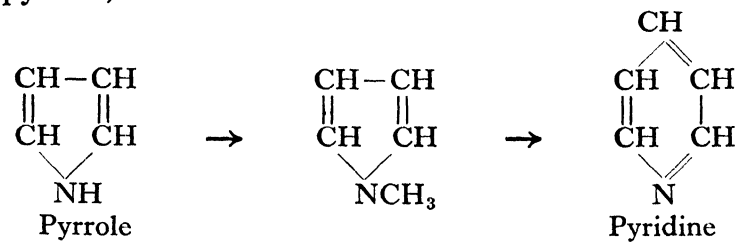
the resulting methylated compounds being then able to undergo intramolecular transformation, by which the methyl group can

* See also Chapter I.

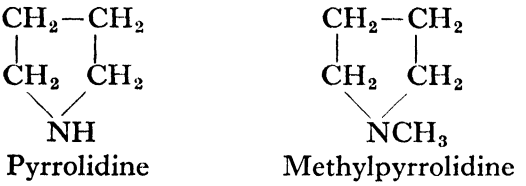
† *Ber. Deut. pharm. Ges.*, 1914, **24**, 35.

‡ *Ber.*, 1907, **40**, 3771.

enter the ring and so produce, for example, a pyridine ring from methyl pyrrole,

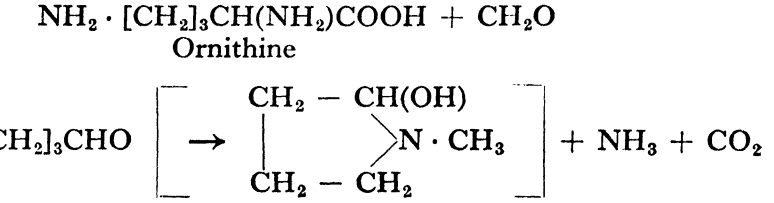


In support of these views, Pictet states that he was able to isolate a number of pyrrolidine bases by steam distillation of the leaves of tobacco, carrot, parsley, and coco. These simple bases, which include pyrrolidine and methylpyrrolidine, he terms photo-alkaloids:



Robinson's views,* which owe their inception to the simple synthesis of tropinone already described, differ fundamentally from those of Pictet. The amino acids and the carbohydrates are regarded as the most likely starting points for the majority of phytochemical syntheses. The chief initial compounds employed are ammonia, formaldehyde, ornithine (p. 144), lysine (p. 144), and the degradation products of the carbohydrates. Citric acid is suggested as the source of acetone residues which it supplies as its oxidation product, acetone dicarboxylic acid. Further, a reactive acetone derivative may be found in diacetylacetone or other polyketens.† One or two of the applications of these suggestions may be briefly considered.

It has already been noted (p. 216) that when formaldehyde is employed for the methylation of amines, oxidation also takes place, amino alcohols yielding methylaminoketones. The reaction of formaldehyde and ornithine might therefore yield a carbinol amine of the pyrrolidine series:



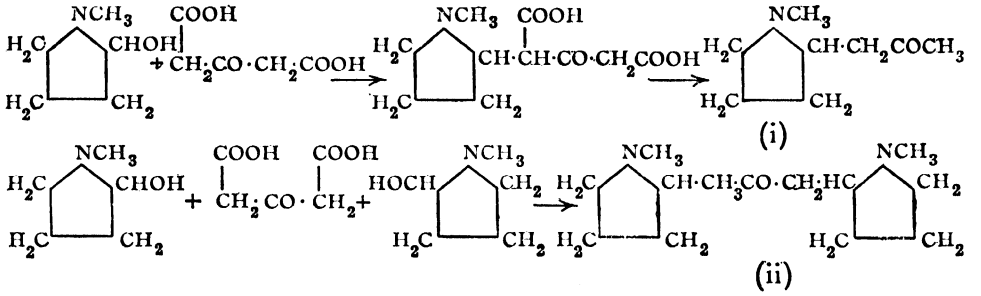
* *Trans.*, 1917, **111**, 762, 876. † Collie, *Trans.*, 1893, **63**, 329; 1907, **91**, 1806.

Further oxidation accompanying methylation might attack both ends of the molecule to give succindialdehyde and methylamine:

$$NH_2 \cdot [CH_2]_3CH(NH_2)COOH + 2CH_2O$$

$$= OCH \cdot CH_2 \cdot CH_2 \cdot CHO \left[\rightarrow \begin{matrix} CH_2 - CH(OH) \\ | \qquad\quad \rangle NCH_3 \\ CH_2 - CH(OH) \end{matrix} \right] + 2CH_3NH_2 + CO_2$$

After condensation with acetone dicarboxylic acid and elimination of carbon dioxide, hygrine (i), cuschygrine (ii), and tropinone (p. 222) are obtained:



(i)

(ii)

The condensation product which forms the source of these alkaloids may also be the progenitor of nicotine by further condensation with formaldehyde and ammonia. Similarly, by the extension of these simple reactions, Robinson is able to account for the formation of the majority of the alkaloids, as well as for the frequent occurrence of several closely related alkaloids in the same plant.

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